MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

VOL. XXV MARCH–APRIL, 1933 NO. 2

ROLAND THAXTER[1]

WM. H. WESTON, JR.

(WITH PLATES 16 AND 17)

In the history of any science the influence of an occasional outstanding man, both through his own work and through the activities of men trained by him, leaves a lasting impression on the field which was peculiarly his own. In Mycology Dr. Thaxter was such a figure, and his death on the 22d of April is a loss deeply felt not only in this field but also in Botany as a whole. Perhaps, because he exerted such a distinct influence, it may be fitting to record here some notes on his life, his work, and his personality, more for the benefit of younger men who, although they have known his work, unfortunately never had the opportunity to meet him, than for his contemporaries to whom he was better known.

Roland Thaxter was born in Newtonville, Massachusetts, August 28, 1858, his inheritance from both his parents being an unusual one. His father, Levi Lincoln Thaxter (Harvard 1843), although trained in Law was by nature and inclination a scholar, an authority on the life and works of Browning, with a considerable reputation in the field of literature, a respected and well loved member of a literary and artistic group comprising such men as James Russell Lowell, Henry D. Thoreau, Thomas Bailey Aldrich, Nathaniel Hawthorne, and William Morris Hunt. His mother, Celia Laighton Thaxter, will always be remembered for

[1] Presented in part at the first meeting of the Mycological Society of America held in Atlantic City December 28–30, 1932.

[MYCOLOGIA for January–February (25: 1–67) was issued February 1, 1933]

her poetry, in which her love of nature and her deep religious feeling were manifest. To the unusual qualities of his parents, to the scholarly and cultured atmosphere of their home, and to their material and spiritual encouragement is attributable much of Dr. Thaxter's character and ability.

In his early years he attended several schools, among them the Boston Latin School, and finally from the private school of Joshua Kendall in Cambridge he entered Harvard in the autumn of 1878, receiving in 1882 the degree of A.B., Magna Cum Laude, with honorable mention in Natural History and English Composition. He was one of the outstanding members of this class of 1882, which among 182 who graduated (227 having started as freshmen), after 25 years comprised five professors and one widely known instructor on the Harvard Faculty and 11 in other universities, and a list of 28 in Who's Who, including, in branches other than academic, notable doctors, lawyers, authors, financiers, politicians, and diplomats. The year after graduation, 1882–1883, Dr. Thaxter himself has described as mostly lost because an accident at the end of his senior year kept him practically on his back for nine months, in spite of which he mentions "work done, mostly biological, in entomology, one or two short papers published." In the autumn of 1883 he entered Harvard Medical School and although, after a few months, imperative duties demanded much of his time at home, yet he passed the examinations and started the second year with his class. Meanwhile, however, he had received the Harris Fellowship, a two-year appointment which enabled him to leave the Medical School and enter the Graduate School of Arts and Sciences, an important step in his career, as he concentrated in research in Cryptogamic Botany under Dr. Farlow, with whom he served as assistant from 1886 to 1888, published his first mycological paper, "On Certain Cultures of Gymnosporangium with Notes on their Roesteliae," in 1887, and in 1888 received the degrees of M.A. and Ph.D. in Natural History, his thesis being a monograph, "The Entomophthoreae of the United States," published the same year. After a brief interlude tinged with plant pathology and applied mycology at the Connecticut Agricultural Experiment Station from 1888 to 1891, he was called back to Harvard to an assistant professor-

ship as Dr. Farlow's associate, and continuing in this capacity took on the full responsibilities of Cryptogamic Botany when in 1896 Dr. Farlow relinquished the instructing of undergraduates although still continuing the guidance of some of the graduate research students. In 1901 Dr. Thaxter became full professor, the position he occupied until 1919, when, after Dr. Farlow's death, he became Professor Emeritus and Honorary Curator of the Farlow Library and Herbarium, having retired voluntarily in order to pursue his own research uninterrupted.

The scope of Dr. Thaxter's work and the extent of his intimate knowledge of the fungi as revealed by the many papers which he published (see list) are most unusual and impressive. To be sure, his monographic work on the Laboulbeniales is his most monumental contribution to mycology, but fundamental and important work on many other groups is evidence of his indefatigable investigations and his wide knowledge. Early in his career he established the Myxobacteriaceae, a new order of the Schizomycetes, characterized by a vegetative phase of distinct, rod-like individuals swarming in masses in a gelatinous matrix, which by their concerted action form fruiting bodies of various degrees of complexity. This life history, resembling somewhat that found in the Acrasiales, was so aberrant and unusual among the Schizomycetes that much controversy resulted among pathologists and mycologists after the publication of Dr. Thaxter's first paper in 1892. Yet the convincing evidence of this paper and later ones of 1897 and 1903, corroborated by other investigators, established the group with certainty so that by now it occupies an accepted place in classification and is included even in textbooks of bacteriology. Furthermore, not only his monograph of the Entomophthoraceae and his comprehensive revision of the Endogoneae but also a series of papers on the Zygomycetes and one on new or peculiar aquatic fungi, comprise a significant contribution to our knowledge of the Phycomycetes. In the case of the Ascomycetes several papers on such unusual genera as *Wynnea*, *Myxotheca*, *Midotis*, *Ionomidotis*, and *Cordierites*, as well as discussions of interesting new species of *Uncinula* and *Taphrina*, together with studies of the perfect stage of *Aschersonia*, are evidence of his interest and investigations beyond his chosen

Laboulbeniales. Among the Fungi Imperfecti a series of contributions describing in detail a considerable list of new and interesting genera such as *Heterocephalum*, *Cephaliophora*, *Desmidiospora*, *Gonatorrhodiella*, and others revealed his knowledge of this vast and perplexing group. In the Basidiomycetes papers on the unusual rust *Maravalia* and the rare phalloid, *Phallogaster*, as well as reports on smuts of onion and other hosts are concrete evidence of his attention to that group. Indeed, although he never published on the Hymenomycetes and avoided study of the fleshy and edible representatives, his knowledge of the more obscure lower Basidiomycetes was very comprehensive, far more than is revealed by these relatively few papers. In addition, his two miscellaneous papers on fungous parasites of living insects not only described many interesting forms new to mycology, but covered a wide range of fungi, chiefly among the imperfect fungi and Ascomycetes, to a lesser degree among others of doubtful affinities.

Although Dr. Thaxter is known primarily for his work in mycology, he was by no means limited to this field alone. He had an unusual knowledge of the algae, and although he published but one paper on "The Structure and Reproduction of Compsopogon" he had made large and well chosen collections of both fresh water and marine forms, some of them worked over in detail by Dr. Farlow, others studied, identified, and then added either to the Farlow Herbarium or to the valuable collections in the Botanical Museum. Similarly Dr. Thaxter had an intimate familiarity with the Bryophytes and although he never published concerning them he had studied them under their natural conditions during his travels and had made extensive discriminating collections that became valuable additions to the Farlow Herbarium and to the collections of various specialists in this field. He had given considerable attention to lichens as well, his knowledge of them was unusual, and although but one of his publications deals with them, the specimens which he gathered with keen discernment during trips to such places as the British West Indies became the basis of papers by Vainio, Riddle, and others to whom the material was generously furnished.

All of his occasional papers, however, he regarded as mere side

issues, referring to them by the derogatory diminutive of "Opuscula," yet these alone would have given him eminence as a mycologist had he never followed his main line of investigation, the monumental work on the Laboulbeniales for which he will be forever renowned throughout the scientific world. The course of this main line of his work follows a historical sequence of development as logical as that traced for the different phases of Pasteur's investigations by Duclaux. His first interest in biology was divided between insects and fungi, and very early he acquired a thorough groundwork in both groups which enabled him to appreciate the importance of the biologic aspects of their interrelationship. Naturally he became interested in the parasitism of insects by fungi, perhaps the most involved and significant phases of the intricate association between these two diverse and extensive groups. It was this field, therefore, that he chose for his doctorate work, and his thesis, the well known "Monograph of the Entomophthoraceae," was not only a most comprehensive and able contribution in itself but also a significant step in the undertaking of his life work. In spite of the phytopathological demands of his first position he was not diverted from his set purpose of extending his studies in this field to the other groups of fungi parasitic upon insects, and it was not long before he began an investigation of *Cordyceps*, *Isaria*, and related forms on which he continued work throughout his life, gathering extensive collections of material and literature, and although he never published a monographic study of the group, his paper on *Aschersonia* in 1914 was a valuable contribution and he was sought as an authority by others whose research lay in the same field. As his investigations reached out to other groups his appreciation of the significance and importance of the field opened up by Leidy's classic "Flora and Fauna of Living Animals" led him to investigate the fungi parasitic in the intestines of insects. After some years he described a few of these fungi in miscellaneous notes, extending Leidy's contributions by descriptions of additional Enterobryeae and related forms. Continuing ever farther in his chosen field he was led into studies of the entomogenous Fungi Imperfecti, work which he continued intermittently for years and on which he published two short but important contributions in 1914 and

1920. Through all these years also he continued his interest and his work in the Entomophthoraceae, being widely recognized as an authority in this group. It is noteworthy that although interested chiefly in such primarily biologic matters as the morphology, development, host range, and distribution of these several groups of entomogenous fungi with which he concerned himself, yet he had a keen realization of the possibilities of their economic application to insect control and was the instigator of successful work in this applied field done by such men as Speare and Rorer.

Early in his investigations he began his study of the Laboulbeniales, a group then practically unknown, and as he developed his technique of collecting, mounting, studying, and drawing these organisms, he soon became increasingly absorbed in them as he found them to be a veritable *terra incognita* opening up the limited yet paradoxically extensive field that was to become his life work. The first volume of his investigations, modestly entitled "A Contribution towards a Monograph of the Laboulbeniaceae," was published in December, 1896, a quarto volume of 429 pages and 26 plates which not only covered the taxonomic consideration of genera, species, and families, but discussed general matters of the morphology and development of these organisms as well as their occurrence, distribution, and relation to the insect host. So complete, so comprehensive, accurate, authentic, and beautifully illustrated was this volume that it at once gave Dr. Thaxter international recognition and the four volumes that have followed since then have augmented this. The second contribution, which appeared in 1908, increased the total number of species and varieties to about 500, and in addition presented further significant facts of development, distribution, and other matters of general interest. Following this, nine preliminary papers added an almost equal number of additional species, while Part 3, which appeared in 1924, extended the same searching taxonomic study to additional families not previously treated in detail. Part 4, appearing in 1926, continued this monographic treatment of the group, increasing our knowledge of these organisms to an extent which may be gauged by the fact that it brings the genus *Rickia*, established on *R. Wassmanni* by Cavara in Europe in 1899 and

formerly comprising but two species, to over 100 species worked out in exacting detail and exquisitely illustrated. Moreover, this as well as the other volumes was a monographic treatment in the broadest sense of the word, being by no means confined to a mere taxonomic discussion of genera, species, and families; indeed, it should be noted that Dr. Thaxter's descriptions of the development were so detailed that the later work of others using serial sections and modern cytological technique have added little to his presentation of the nuclear conditions involved, while his discussion of the different sex conditions of unisexuality and bisexuality involving self fertilization and cross fertilization are almost prophetic in their fundamental grasp of conditions which recent work has shown to exist in other groups.

When working on Volume 5, which was published in 1931, Dr. Thaxter planned to complete the series in this volume with a systematic treatment of the species which had been collected and described since earlier parts appeared, and a final consideration of host range, distribution, and other points of general interest. The number of species proved to be so large, however, that he was forced to restrict the number of illustrative figures to 1136, comprised in 60 plates, to limit the specific descriptions, and to defer the general discussions. In 1932, therefore, he was at work on the additional species of the genus *Laboulbenia* whose number had precluded their inclusion in the previous volume, had completed many of the illustrations, and was planning with indomitable courage despite increasing handicaps to include the treatment of this large and difficult genus in a final sixth volume with addenda, a general revision of the classification, a host index, and the consideration of the long deferred matters of general biologic interest.

To all who have followed this monograph through these several parts, the tragedy that death cut short his work before he had completed his general consideration of the group in its more general biologic aspects, is an appalling one. This knowledge he alone possessed. Others, with his monograph to help them, undoubtedly can deal satisfactorily with additional species that may be encountered in the future, but the treatment of the group as a whole from its biologic aspects he alone could accomplish.

Through his untimely death it is forever lost. This monograph, the main activity of his life, developing as the logical outcome of his interest in fungi in relation to insects, stands out as one of the greatest single pieces of work of all time in mycology, a monument to his courage and devotion as well as to his productive scholarship.

All of his work, whether his several occasional papers on fungi, his few but important phytopathological reports, or his monographic studies of the Laboulbeniales, was characterized by exhaustive thoroughness and completeness in the work itself, the most exacting accuracy of illustration, and a most effective and well ordered presentation. Yet the student, expecially the beginner in mycology, encounters certain difficulties in Dr. Thaxter's publications. Because of his aversion to short, disconnected sentences and his conviction that the style of Thackeray was best suited to scientific exposition, his writing was not always easy to follow; while in his desire to avoid misrepresentation through statements too sweeping and inclusive he was led at times to qualify his statements so cautiously that their interpretation required undue labor on the part of the reader. Also following the earlier custom he gave references in brief footnotes rather than in detailed lists of literature cited, nor did he append to his papers summaries emphasizing the main points comprised, while with his illustrations he never included an absolute scale that would permit comparison of their essential measurements.

In his teaching Dr. Thaxter was at his best with graduate students whose purposive interest and eager enthusiasm for investigation he developed and guided most effectively from his extraordinary knowledge of the fungi and his extensive store of material and literature. He was an exacting master under whom to work, but no student could resent what was demanded of him in faithful adherence to work, painstaking conscientiousness of execution, unremitting accuracy in drawing, and brevity and clarity in writing, because of the realization that Dr. Thaxter was even more exacting and more demanding of himself. In lecturing he was neither fluent nor vivid nor of compelling magnetism, but his direct, sincere presentation and his impressive knowledge of the subject more than made up for any lack of

popular appeal and left a lasting impression even on some of the most uninterested of his classes. Moreover, although his lectures were full of valuable information they were not without occasional gleams of dry humor, of which evidence may be found here and there in notes taken by discerning auditors. In connection with his lectures he used a very condensed syllabus, crammed with facts, closely typed, and reproduced by hektograph with a few of his own illustrative diagrams. Some of us still cherish this outline, and the pages, crowded, faded, and hard to follow, are yet remarkable for the amount of pertinent information they presented in a minimum of space. He always began each lecture with a brief resumé of the previous one, a valuable aid to the student who had missed some points, and for this recapitulation he used the syllabus as a basis.

In the laboratory work of his classes he had to depend on assistants to some extent, especially in the larger general course in Cryptogamic Botany which at times contained 60 to 70 students, but none the less, whether in this or in the smaller, more advanced courses, he always managed to spend some time with each man, and his method of leading the student by skillful questioning in a sort of Socratic dialogue until through the microscope the essential features of structure and development were observed and interpreted correctly, was a revelation in teaching to his assistants who had seen in operation elsewhere the method of providing a detailed laboratory manual which described and illustrated just what the student was expected to work out for himself. It was in the laboratory that Dr. Thaxter was most effective as a teacher, and his training, which developed in his students qualities of intelligent observation and independent investigation, greatly influenced their own research and teaching in later years. In thus training the student, necessarily he emphasized the development of manual dexterity in such delicate manipulations as would reveal hidden details of structure; and as he himself had unusual dexterity, he regarded as unfortunately handicapped that type of student who was quick to absorb recorded knowledge verbatim from lectures and reading, but clumsy and helpless in attempts to accomplish the necessary simple adjustments of the microscope and easy manipulation of material

that would have enabled him to work out things for himself. Of one such student he once said with a sigh, "That man! He never takes a note yet he remembers everything of the lectures, but in the laboratory he hasn't hands, he has hooves."

In Dr. Thaxter's years at Harvard nearly a thousand students worked with him either as undergraduates in one or more of his courses covering general Cryptogamic Botany or the fungi, algae, and bryophytes, or as graduates taking advanced work in research in some of these fields. How great an influence he had on these men may be judged from their enthusiastic letters on the occasion of Dr. Thaxter's 70th birthday in 1928. One student, at that time a professor of English, wrote, "Well do I remember how I stumbled along in his Cryptogamic Botany and how patient and painstaking he was toward me. Even such a dub as I was could not help but realize that he was coming in contact with a remarkable scholar in Dr. Thaxter." Another, then a successful lawyer, wrote, "I think the training I got under Dr. Thaxter was as valuable as any I ever received. With him one learned what it meant to plow a furrow long, straight, clean, and deep in a field of scientific endeavor. With him also one became aware that all organisms are interesting, whether they have legs or not."

To the teaching of Botany it is a great loss that Dr. Thaxter never made available in text-book form his extensive knowledge of the Cryptogams as a whole, or especially of the fungi. His familiarity with the work which had been published in this field and with investigations as yet unpublished but known to him through his extensive correspondence, especially equipped him to accomplish this, and in addition his extensive intimate first hand knowledge of the fungi from his own work furnished him the very requisite which so many who have produced text books that are excellent compilations unfortunately have lacked. Moreover, although he was decidedly opposed to the exploitation of science in popular (and remunerative!) radio talks, lectures, or articles, he had the faculty of writing not only authoritatively but also interestingly and entertainingly, as his account of the Antarctic beech forest and his article on the Farlow Herbarium amply demonstrate. He was urged repeatedly to write a text book but he always replied that he had too much to accomplish and that

the time left him would be too short for him to finish his "job" on the Laboulbeniales. The loss to the teaching of botany resulting from his tenacious devotion to his set purpose is more than balanced by the resulting gain to productive investigation in mycology.

Although he never called attention to his travels by publishing miscellaneous lists of his collections from various parts of the world, and although he never took part in any widely publicized expedition he had traveled far more extensively than is generally realized. Beginning while he was still a student, in 1885 he made a botanical and entomological trip to Newfoundland, spent the summer of 1886 collecting in the White Mountains, and in 1887 collected extensively in the mountains of North Carolina and Tennessee. In 1890 he traveled and collected in Jamaica, in 1897–1898 he devoted his sabbatical leave to study and collecting first in Florida and then in Europe, and in 1900 he again went to Europe for further travel and investigation. The year of 1905–1906 he spent in South America, sailing from Liverpool in August for a month in Buenos Ayres, thence traveling via the Faulkland Islands and the Straits of Magellan to Chile for three months in Santiago, Concepcion, and Corral, revisiting the Straits of Magellan for a two months' stay at Punta Arenas in the region of the great Antarctic beech forest, and finally, after seven weeks in the Argentine, returning to Liverpool and thence to Cambridge in June, 1906. Again on sabbatical leave in 1912–1913 he visited the British West Indies, spending some time in Grenada in the mountains above St. Georges, where he made extensive and valuable collections and carried on his work indomitably despite exceedingly unfavorable circumstances, continual torrential rains, constant inroads of destructive moulds and insects among his specimens, exceedingly primitive living conditions, and recurrent illness through which he lost twenty-four pounds in a few weeks. From there, after a brief collecting trip to Tobago, he went to Trinidad where at the hospitable home of J. B. Rorer he regained his health and enjoyed six months of active successful work. It was toward the end of this trip that he wrote Dr. Farlow, "I doubt greatly if I tempt the tropics again for they do not use me well however much I like them."

Although he did not publish lists of collections from such trips and had a horror of the hastily compiled lists and half popular, half botanical travelogues which result from the forays and trips of some botanists, yet his letters to Dr. Farlow and others while on such trips are fascinatingly complete accounts of the cryptogamic flora of the regions which he investigated, replete with information as to the occurrence, location, and conditions of growth of unusual and interesting forms, abounding in vivid, picturesque descriptions of the country and its plants, and touched here and there with a deft and dry humor that made such letters not only authoritative but most interesting accounts. Moreover, he was never content merely to collect material and after hastily examining and listing it, dismiss it to oblivion in his herbarium. Rather, he persistently worked over his material with great thoroughness so that his accumulated collections thus became part of his own great store of knowledge, although unpublished. As a result the extent of these collections and their value to mycology may not be realized for years until, having been made part of the Farlow Herbarium, they are again worked over by others in detail. Always, in his teaching and in his own investigations, he showed keen appreciation of the desirability of first-hand knowledge of fungi and other organisms, not only in the laboratory but also as they grow under natural conditions in the field.

Although perhaps thought of by some as a laboratory investigator because in later years much of the material for his monograph of the Laboulbenias was sent him by collectors in different parts of the world, he was ever a persistent, keen, discriminating collector and kept up his field work until relatively recently. All of the environs of Cambridge he visited with his precisely equipped collecting basket, usually alone although at times accompanied by assistants or friends, either on his bicycle or on foot in the 1890's, or later by trolley, train, or bus, so that he came to know the possibilities of the regions within ten to twenty miles most exactly. He could tell his assistants the very places in the clay beds of the brickyards where *Ricciocarpus natans* and *R. fluitans* could be found in abundance, where *Marsilia* occurred, and where *Anthoceros* could be secured.

the exact shores of ponds in Winchester and Arlington where *Isoetes* might be found, and the very pools of the Mystic River tide flats where *Beggiatoa mirabilis* developed in profusion. Of the haunts of many less known organisms which the ordinary collector fails to discover, he had an intimate first-hand knowledge and he felt keen regret when these were destroyed in the real estate developments inevitable to all suburbs,—the exact pool at Stony Brook where *Araiospora pulchra* occurred, now, alas, filled in by refuse from a nearby stone-crusher, the particular ditch near Lexington where *Monoblepharis insignis* was found, now dry and barren, and the swamp near Fresh Pond where species of *Rhipidium* could be secured on baits of green apple or pear, now filled in with rubbish and supporting a spur track, a laundry, and two gasoline stations. With equal thoroughness he knew the mycology of the more secluded and less invaded territory around Kittery, which through his collections has become the *locus classicus* for many interesting and important species. In his field work he demonstrated conclusively that very remote, inaccessible territory is not the only source of rarities and that important findings reward discriminating and intensive search near home.

Perhaps the outstanding characteristics of his distinctive personality were his stern, undeviating loyalty to his work and to its ideals. Day after day he would be at the laboratory perhaps as early as seven in the morning, and in the afternoon would continue working usually until six o'clock or after. These long hours of work and the tremendous amount of productive investigation he accomplished were carried on in spite of persistent hindrances of illness that would have been insurmountable for any but his unyielding tenacity. Being himself thus wholly devoted to his work, it was only natural that he should expect the same of others, and the example of his unremitting devotion was a stimulus to all those who came in contact with him.

In him also, as in many other great characters, there was a certain grim and indomitable adherence to his chosen course and to his ideals. He was an austere disciplinarian, believing in the salutary effect of discipline on the development of character, a quality some of us have especial occasion to remember. For

example, while becoming familiar with various groups of fungi as a first year graduate student under Dr. Thaxter, I early developed an interest in the Phycomycetes, and on one occasion, after working out and identifying a difficult Pyrenomycete which Dr. Thaxter had given me, injudiciously remarked that I disliked that group and far preferred the Phycomycetes; whereat Dr. Thaxter replied that one with as little knowledge of fungi as I, was hardly qualified to express preference for one group over another, and for weeks thereafter kept me busy working on nothing but Pyrenomycetes. Realizing that enthusiasm had overstepped discretion, and remembering vividly that Dr. Thaxter had once told of forcing himself to work out a set number of problems each day for months until he had mastered a certain phase of mathematics, I devoted myself to Pyrenomycetes.

Although austere and reserved, Dr. Thaxter had a keen appreciation of beauty which was manifest in his love of music, art, and literature. He was an accomplished musician, admiring greatly the formal compositions of Brahms and Beethoven, though looking a little askance at Sibelius and Rimsky-Korsakov. He showed a discriminating appreciation of beautiful works of art and architecture, and he loved good books which sometimes he would read to his friends most effectively in his resonant and expressive voice. Indeed, knowing his sensitiveness to beauty, one could not help but feel that certain groups of fungi attracted him not only because of their scientific interest and importance but also because of the diversity and exquisite nicety of form and structure which they revealed.

Moreover, although not characterized by lightness of heart or irrepressible wit, he had a dry sense of humor which from time to time revealed itself to those associated with him. Once, for example, when several of us, as graduate students, were working in the old laboratory in the top floor of the University Museum, our ranks were increased by a botanist from the West who, in one year's leave, was endeavoring to increase his botanical background by several courses at Harvard. In Botany 20b his lack of knowledge of the fungi was outweighed by his enthusiasm, and when one day *Aspergillus clavatus* appeared in a culture he was much intrigued by this striking organism, and on being told

by his fellow workers that it was an *Aspergillus*, he hurriedly pushed past the partly closed door of Dr. Thaxter's sanctum and announced, "Professor, I have *Aspergillus* here"; whereat Dr. Thaxter, looking up at the unexpected and precipitate visitor, remarked drily, "Ah, yes, *Aspergillus*,—not a rare genus, but an interesting one," and turned without further comment to his Laboulbenias.

His letters also were enlivened by deft and whimsical characterizations of people and situations and enriched by frequent humorous touches. In one letter, for example, acknowledging the receipt of a manuscript destined for the Annals, he enclosed a portion of the envelope in which it had been sent, revealing the student's legend, "Please Do Crush or Fold." with the comment that the post office people as usual had failed to heed the direction on the package.

He was a man of great dignity and self restraint, which gave his quiet comments and suggestions added force. His poise remained undisturbed by such laboratory calamaties as the burning out of an autoclave or the failure of lights from blown fuses and carried him unperturbed through unexpected and upsetting occasions.

He had of course certain prejudices, perhaps the most outstanding being his prejudice against smoking. Aside from the fact that he regarded the odor of tobacco smoke as distasteful and unpleasant, he had a deep conviction that smoking would impair the delicate control of the hand necessary for the accuracy of drawing which he regarded as an indispensable essential in mycologic work. For the most part this prejudice affected his students but little, as the habit was not so general as it is now, and no smoking was allowed anywhere in the Museum Building. Students who did smoke habitually, however, sooner or later were made vividly aware of Dr. Thaxter's feelings in this matter. A lecture on the evils of tobacco to one such student whose excellent illustrations of entomogenous fungi have since aroused the admiration of mycologists, led as a sequel to one of the few times when Dr. Thaxter was ever actually taken aback. Late that afternoon, Dr. Thaxter, who was demonstrating a point in drawing to one of the neophytes, remarked, "Well, I do not know

what's the matter with me. My hand is not steady today," whereupon the student recently reproved, bending over his work in the far corner of the laboratory, remarked in a penetrating whisper, "nicotine!" at which Dr. Thaxter laid down the pen and without a word retired to his office.

He had a decided scorn for illogical forms of simplified spelling, as students submitting manuscripts to him speedily found out. He had little patience also with lack of conscientiousness in any form, whether in the careless, inaccurate illustrations that reflect inadequate observation and hasty execution, or in the vague writing whose lack of clarity in expression usually denotes lack of clarity in thought.

He was a member of Phi Beta Kappa, of the Botanical Society of America, the American Phytopathological Society, the Boston Society of Natural History, the American Philosophical Society, and the National Academy of Sciences, a fellow of the American Association for the Advancement of Science and of the American Academy of Arts and Sciences. His eminence was recognized formally by his colleagues and fellow botanists, as he was elected President of the New England Botanical Club, President of the American Mycological Society, and President of the Botanical Society of America. This recognition extended to Europe as well, for he was an honorary member of the Russian Mycological Society, the Linnean societies of London and of Lyons, the Royal Botanical Society of Belgium, the Royal Academies of Sweden and Denmark, the Botanical Society of Edinburgh, and the Academy of Science of the Institute of France, as well as the only American botanist of his time on whom honorary membership was conferred by the British Mycological Society and the Deutsche Botanische Gesellschaft. He was until his death the American editor of the Annals of Botany, being appointed in 1907 to succeed Dr. Farlow. For some of his early work on the Laboulbeniales he received the Prix Desmazières from the French Academy.

Less formal but even more widespread evidence of his eminence is found in the unusual reputation as a mycologist which he bore among botanists throughout the world. This was the result not only of his publications but also of the general recognition of his

extraordinary knowledge of the fungi among the great numbers of investigators who turned to him for help in puzzling questions as to the identification and nature of obscure and perplexing organisms. Workers not only in mycology but in many other branches of natural science sought the benefit of his knowledge and the amount of correspondence he carried on with those who consulted him during the last forty years seems appalling to us lesser men of the present, especially when we realize that the whole great bulk of his correspondence was carried on without the aid of any secretary or typist, most of it being written in his own hand, for he had a feeling that typewritten letters were in a sense too mechanistic and impersonal for most correspondence, the others being typewritten by himself on a small portable typewriter. That many botanists were aided by his expert knowledge and sound judgment in mycological matters is obvious from the voluminous correspondence that he has left. It is certain also that many were disconcerted through his uncanny familiarity with fungi which enabled him to identify as insect eggs the specimens they had sent as Myxomycetes, to set their minds at rest that their new genera of leafspotting fungi in reality were the adherent sporangia of *Pilobolus*, and to tell them in detail the structure, development, and relationship of "new" forms they had discovered from his own exacting work on these same forms twenty years before.

Many of Dr. Thaxter's letters were written to mycologists and other botanists, especially those who had just begun to publish, concerning their papers which Dr. Thaxter, having read with conscientious care, commented upon at length, encouraging or criticizing impartially and from his greater knowledge bringing out points of significance and interest untouched in the paper itself. Many a young investigator having published his first paper was cheered and heartened by this encouraging recognition from Dr. Thaxter, and many also were stimulated to more careful work and more painstaking illustration by his impersonal and just criticism.

Those of us who had the privilege of being associated with him could sense beneath his reserve and austerity a kindliness which revealed itself here and there in phrases in letters, in a

turn of conversation, in unexpected and much prized gifts, or in unexpected help through difficulties. To see the genial whimsicality that he showed toward the dog, Bobby, or toward the parrakeet, Malatesta, or to watch the solicitous care with which he tended some of the rare and beautiful flowers that he grew in his garden and in the house with such success, was to glimpse another side of his character and to appreciate that he was a human and likable man as well as the greatest mycologist of his time.

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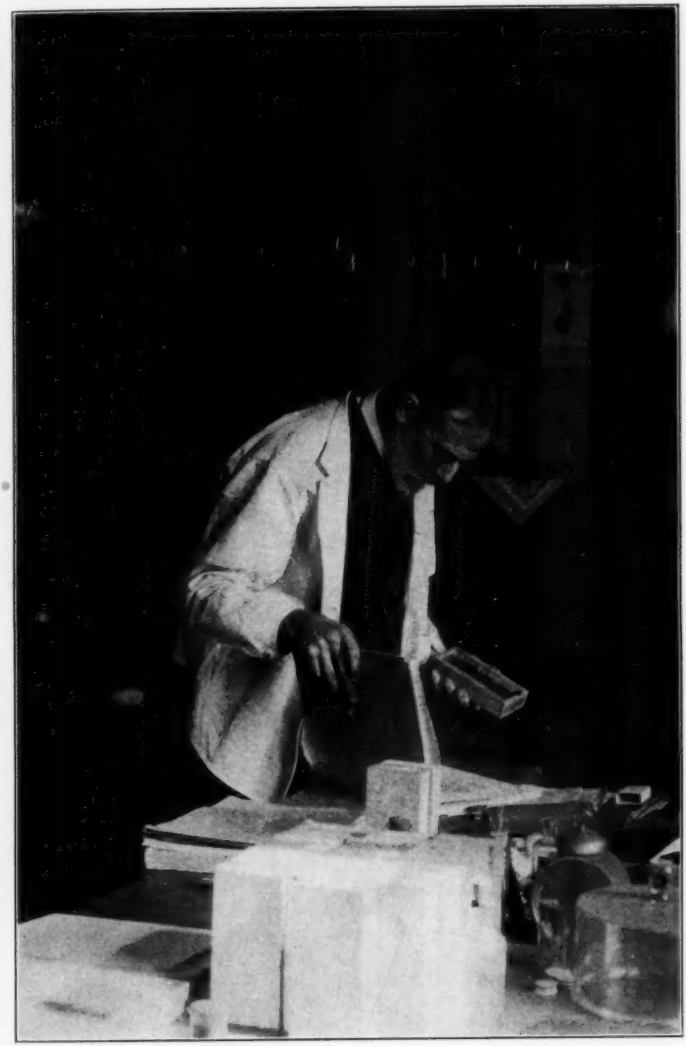

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THE PERITHECIUM AND ASCUS OF PENICILLIUM[1]

B. O. Dodge

(WITH PLATES 18 AND 19 AND TWO TEXT FIGURES)

Brefeld[2] began studying the fungi at the time when the question of pleomorphism was exciting considerable interest among mycologists. *Penicillium* was the most common mold, and he determined to learn whether it was also, like *Aspergillus*, pleomorphic, having a sexual stage as well as the conidial stage which was familiar to every one. Brefeld was not satisfied to base his conclusions on cultures obtained by mass transfers of conidia. He adopted the new method of at least starting with single spore cultures. The great detail and beauty with which he described and figured the various steps in the development of the ascocarpic and conidial stages of his fungus were so convincing that his story of "*Penicillium glaucum*" became the favorite for illustrating the life history of ascomycetes.

One might well question whether Brefeld in identifying his fungus as *Penicillium glaucum* had the fungus described by Link. Furthermore, no one today, according to Thom,[3] seems to know exactly what fungus Brefeld himself had. It would serve to clarify the situation, however, if we could compare the stages in growth observed by Brefeld with what we would find in studying a species that has the same kind of ascocarp. Such a species has been found recently, and, in following its growth in culture, one realizes, if he makes due allowance for the state of mycological knowledge in Brefeld's time, how beautifully he illustrated the developmental features as he understood them.

Dr. Rhoda Benham of the Department of Dermatology, Col-

[1] Presented at the first meeting of the Mycological Society of America held in Atlantic City December 28–30, 1932.

[2] **Brefeld, O.** Botanische Untersuchungen über Schimmelpilze. II. Die Entwicklungsgeschichte von *Penicillium*. 1–98, 1874.

[3] **Thom, C.** The Penicillia. 1–644. Baltimore. 1930.

lege of Physicians and Surgeons, has recently been making a study of the fungi found inhabiting the skin and alimentary canal of 100 young men and women. She was particularly interested in species of *Cryptococcus* and other yeast-like forms related to *Monilia albicans*. She will report on her work later. While making this survey she also obtained cultures representing dermatophytes and other species of fungi, most of the latter being saprophytes. We are indebted to her for several hundred such cultures and to Dr. C. W. Emmons for some stained sections of perithecia which were turned over to the writer for study and for exhibits at The New York Botanical Garden. One culture, Ed 24, proved to be a species of *Penicillium* which was producing perithecia abundantly. No similar species is mentioned by Thom [3] but upon examining our cultures Dr. Thom suggested to the writer that the fungus was near *P. javanicum* van Beijma, probably a variety of that species. If one grows the strain representing van Beijma's fungus obtained from Dr. Thom along with our Ed 24 strain he has no difficulty in distinguishing the two forms regardless of what culture medium is used. If he were to rely on van Beijma's description and figures he would certainly separate the two forms specifically. For example, she says the asci are 4–6-spored. Ours are 8-spored. Her cultures are dark-orange and the perithecia are orange-colored. No such yellow or orange colors are shown by our Ed 24. The asci and ascospores are noticeably smaller than ours, although the extreme measurements given are about the same. Any one can distinguish the two species by comparing mounts. The asci of *P. javanicum* average about two or three microns smaller than ours. The ascospores average at least a micron smaller. In other morphological features of the ascocarpic stage they are very much alike. Some may prefer to consider them merely as varieties of a collective species. This would require either varietal names or some designating numbers, but would be fully justified if, by starting with single ascospore cultures of one variety one could obtain a form morphologically and culturally indistinguishable from the other variety and vice versa. What would happen if the same method of study of the ascocarpic stage were applied to the several hundred other species of *Penicillium* created on the basis of their conidial stage alone, would be interesting to see.

Penicillium Brefeldianum sp. nov.

Mycelium and conidial masses variously colored depending first on the particular race and second on the nature of the culture medium, whitish, cream, peach, fawn, mouse or grayish to pale green; on corn meal agar sparse with few conidia; homothallic.

Conidia spherical to short elliptical smooth, 1.5–2 × 2–3 μ; penicillus commonly monoverticillate; sterigmata 2.5–3 × 7–10 μ, the spore-forming tube prominent; conidiophore short, slightly enlarged at the tip, side branches rather frequent, 3–4 × 5–150 μ.

Ascocarps spherical, whitish to pale tan, non-ostiolate, superficial, growing upon, and more or less surrounded by, a loose weft or network of hyphal branches, 100–200 μ in diameter, mostly about 150 μ; asci oval, pear-shaped to spherical, 7.5–12 × 10–15 μ, 8-spored; ascospores globose to slightly elliptical, hyaline, finelv echinulate, 2.5–3.8 × 3–4 μ.

Isolated from alimentary tract of human.

Culture deposited in the Herbarium of The New York Botanical Garden.

Conidia e sphaericis breviter elliptica, laevia, 1.5–2 × 2–3 μ; penicillus univerticillatus; sterigmata 2.5–3 × 7–10 μ; conidiophorus asymmetricus, ramis lateralibus non nullis, 3.5–4 × 5–150 μ.

Perithecia sphaerica, albida vel pallide brunnea, exteriora, 100–200 μ; asci ex ovalibus sphaerici, 7.5–12 × 10–15 μ, 8-spori; ascosporae e globosis subellipticae, hyalinae, minute echinulatae, 2.5–3.8 × 3–4 μ.

Brefeld worked merely with clean cultures and he could not have carried his single spore culture pure long enough to show all of the stages in the life cycle from ascospore to ascospore. He thought that for the maturing of the ascospores it was necessary to grow the fungus finally on bread in dark cool places shut off from the air. Therefore, he would carry his cultures several weeks to the sclerotial stage and then sow the sclerotia on thin slices of bread pressed between glass plates to exclude the air. Brefeld has pointed out how nearly the ascospore, which he figures with a furrow running around the equatorial region, resembles ascospores of *Eurotium* (*Aspergillus*). It was by the germination of these ascospores, however, and the formation of *Penicillium* conidiophores directly connected with the ascospore

germ tubes that convinced Brefeld that he had followed out the life history of his *Penicillium.*

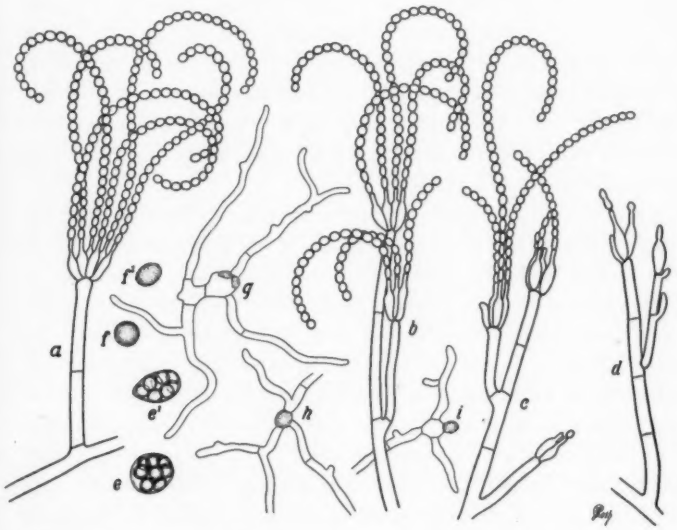

FIG. 1. *Penicillium Brefeldianum.* a–d, various stages in the development of the conidial stage; e, e^1, spherical and oval asci; f, f^1, spherical and elliptical ascospores; g–i, three types of ascospore germination, g showing fragments of the spore-wall clinging to the germ vesicle as figured by Brefeld.

SINGLE SPORE CULTURES

From Dr. Benham's culture, Ed 24, twelve single conidium cultures were isolated and grown on corn meal agar. In every case ascocarps began to appear after about two days. Asci began to form by the end of seven days and ascospores were visible within ten days from the time the cultures were started. In not all cases, however, do the ascocarps mature in such a short time. This will depend upon the conditions of the medium and the temperature of the room.

Ten single ascospore cultures were also isolated. Germination was abundant after about ten hours. Two or three germ tubes are formed. If the ascospores are young they germinate without much swelling, pushing out germ tubes directly (FIG. 1, h); or

the spore contents may move out as a vesicle from which the germ tubes then emerge (FIG. 1, i) as in *Neurospora*. After the spore becomes fully matured with a thickened wall, it may swell to about twice its original size on germinating, bursting the wall into two parts which cling to the spore (FIG. 1, g) much as is shown by Brefeld in his plates 7 and 8. There is no suggestion of a furrow, however, running around the spore, and the two parts into which the spore wall is usually broken up do not represent or resemble two halves or equal valve parts of a spore wall as is the case in *Aspergillus*.

Culturing *P. Brefeldianum* on agar plates, very definite examples of sectoring were met with. A less pronounced illustration of this is shown incidentally in plate 18, d. Several series of transfers were made from snow-white sectors with few conidia, from fawn-colored sectors, and from pale greenish sectors with many conidia. Very different pictures were presented by the three sets of cultures, each grown separately on Czapek, Sabouraud, dextrose, and potato dextrose agars. On corn meal agar these differences were not so noticeable. The van Beijma strain, when grown on corn meal agar, was strikingly different from any of our strains. Certain of our races produced a greater abundance of conidia and ascocarps. Other races produced many conidia but very few ascocarps. Corn meal agar is the most suitable for the study of ascocarps because of the sparseness of mycelial hyphae and conidia (PLATE 18, a). Other media may produce more ascocarps than appear on corn meal agar, but there is usually in such cases an excessive surface growth (PLATE 18, b–d) in which the ascocarps become imbedded.

On corn meal agar the conidiophores are not at all fasciculate and no coremia have ever appeared on any of the different media. What would happen at low temperatures was not determined. The conidial stage (FIG. 1, a–d) may belong to Thom's asymmetric group. It is certainly not a biverticillate species and it can not belong to the *P. luteum* group, although on Czapek's medium yellow droplets appear over the surface of the culture (PLATE 18, d).

ORIGIN OF THE ASCOCARP

Brefeld's figures of two short bodies, arising from adjacent cells, and coiling spirally about each other as the beginning of the

perithecium are familiar to all botanists. If these coiled organs are the very first indication of where an ascocarp is to arise, then our species is different from the one he worked with. In our fungus one sees first a very short branch arising vertically from an ordinary mycelial hypha at the surface of the medium. This stalk forks, giving rise to two or three primary branches which in turn put out rather stiff secondary branches at broad angles (FIG. 2, a), giving a miniature picture of a small defoliated fruit tree. There is absolutely nothing resembling sex organs or ascogonial coils at this stage. Later on, however, there appear in the crotch or fork of the system rather thick structures irregularly wound or clumped in a loose knot, and which stain more deeply with aceto-carmine. This may be the point at which Brefeld began his study as he says that the coiled bodies arise from a rather thick much jointed mycelial hypha. Crushed mounts stained with aceto-carmine do show such "jointed hyphae" at this stage, but I have not made out the pair of coiled structures figured by Brefeld.

In the Ascobolaceae, Sordariaceae and *Pyronema* one sees first of all the sex organs. There is at least an ascogonium present at the beginning. In *Penicillium Brefeldianum* it is the little hyphal tuft which first appears, and on, or within, which the stromatic as well as the ascogenous elements of the ascocarp arise. It continues to branch loosely growing up around the young perithecium (FIG. 2, b) remaining attached to the base of, but never becoming actually incorporated with, the fruit body as a part of the wall. By compacting its weft of branches in cutting sections by hand one might be misled into thinking that there was a loose outer wall to the perithecium that sloughed off, as figured by Brefeld for his fungus, although he says he was not certain as to the origin and function of this outer weft. One sees the exact relationship if he removes a perithecium, placing it in a drop of aceto-carmine or any mounting fluid. The loose network can be removed with a pair of fine needles. It represents the original subicular tuft which is still firmly attached to the base of the fruit body. Most of the nourishment for the growing perithecium must come up through the original stalk cells. In *Aspergillus* this single hyphal stalk often supports the

perithecium. In our *Penicillium* the perithecium settles down on the agar so that branches of the hyphal tuft may adhere to the basal part. The tangle of free ends forms a fuzzy complex of protecting hyphae. Crushed mounts always show a few scattering brownish amber-colored hyphae adhering closely to the wall of the perithecium. Sections (PLATE 19) do not show that these peculiar cells grow out into the air as a fringe. The end branches of the hyphae composing the fuzzy and rather more compact tuft in the van Beijma strain are always covered with a thick amber-colored crystalline deposit, very noticeable when grown on corn meal agar. These are absent in case of our strain Ed 24.

THE ASCOCARP

During the first seven or eight weeks in cultures on bread, Brefeld's *Penicillium* developed little sclerotia with the three or four outer layers of cells thickened and hardened. They looked like little yellowish grains of sand. After their resting period, they could be induced to develop into perithecia by sowing them again on sheets of bread placed between glass plates. The ascogenous hyphae which had been growing slowly during the first period now increased in length at the expense of the inner tissue of the sclerotium. He saw three kinds of hyphal elements. First, the large septate hyphae; second, the true ascogenous hyphae with peculiar short branches with recurved tips; and third, the very thin hyphae which he said must function to disorganize the inner tissues of the sclerotium to obtain nourishment for the asci. It sometimes required several months to complete the life cycle.

In our cultures of *Penicillium Brefeldianum* on corn meal agar no resting sclerotial stage has so far been observed. Such bodies might develop if certain races were grown on less favorable media. The ascocarp grows on rapidly from the beginning, forming a solid mass of pseudoparenchymatous tissue with little or no differentiation except for the somewhat thicker walls of the cells of the outer layer. At the center will always be found the ascogenous system much as described by Brefeld. The fruit body at no stage is "hard and stony like a grain of yellow sand" although sections (PLATE 18, f) often suggest sclerotioid tissue, and no

doubt unfavorable media might result in throwing the young perithecium into a resting condition where it would become sclerotized. The young perithecia of *P. javanicum* are somewhat tougher than are ours, and tend more to resemble sclerotia by their hardness. Some sections of *P. Brefeldianum* show compact perfectly normal thin-walled cells composing the pseudoparenchymatous stromatic tissue (PLATE 19, b). In every case the ascogenous system occupies the central region of the fruit body, gradually providing a cavity by disorganization processes. Brefeld's description of the growth of the ascogenous system in his species is very complete, and would, except as to the method of the origin of the asci, apply, for the most part, to *P. Brefeldianum*. The older the ascocarp under good growing conditions the larger the cavity and the greater the number of asci with a corresponding thinning out of the sterile wall layers.

FORMATION OF THE ASCUS

The idea is prevalent that the asci of *Penicillium* always arise along curved tubular non-septate hyphae by series of swellings and partial constrictions, so that even when mature no cross walls separate adjacent asci. The method of formation of asci in chains like monilioid conidia or the budding out of one ascus directly to form another would be entirely different from that known for higher ascomycetes. I have, therefore, given this question particular attention and have found the idea quite erroneous.

Just as Brefeld describes so well there are three kinds of hyphae occupying the central region of the young ascocarp of *P. Brefeldianum*. First, there are rather large hyphae with blocky cells with little granular contents. These seldom branch and are not much curled or contorted. They represent ascogonia, perhaps, in function. Second, from hyphae of the first sort arise the more deeply staining primary ascogenous hyphae with granular contents. These are more twisted and irregular in form. Septation is frequent though not always easily made out. Short thick branches, often divided and recurved at the tips, arise at intervals. Third, one sees the system of thinner hyphal threads. These would be Brefeld's digesting hyphae, though to assume that this is their sole function would be erroneous, as will be noted below.

The first asci arise as short side or terminal swellings from the thick primary ascogenous hyphae (FIG. 2, c–g). A septum soon cuts off the young ascus from its stalk or from the protuberance extending from the cell giving rise to the ascus. One after another the cells along an ascogenous hypha bud out to form a branch or an ascus (FIG. 2, d, f). This method of origin is readily made out by crushing perithecia from cultures of *P. javanicum*. Van Beijma [4] was the first to notice this for she says that when one crushes a perithecium the fertile hyphae with asci budding from both sides are pressed out as a skein. If the culture is old and rather dried out prematurely, one sees asci carrying short stalk fragments floating around everywhere, proving further that in *P. Brefeldianum* and closely related forms like *P. javanicum* asci are not commonly borne in monilioid chains. On one or two occasions, however, it was possible to demonstrate very clearly that the adjacent cells of an ascogenous hypha from young ascocarps had rounded up to become asci directly (FIG. 2, h). One often sees in crushed mounts what at first appears to be a few asci in a chain, but by tapping the cover glass, the cluster turns over and it becomes clear that the asci are all borne on short outgrowths from adjacent cells. Preliminary studies of *P. bacillosporum* having the type of perithecium characteristic more of *P. luteum* show, however, that there are species where many of the first formed asci arise from very short twisted chains of cells much as Brefeld figures. A proliferating crosier system here is not an impossibility. As the cavity in the perithecium of *P. Brefeldianum* increases in size the ascogenous system consists more and more of the very thin hyphae growing around irregularly in all directions. They certainly bear asci very frequently (FIG. 2, i).

Emmons [5] has recently proved that in *Thielavia Sepedonium* each cell of the large ascogenous hyphae is uninucleate. This nucleus divides, one daughter remaining behind, the other moving out into a side bud which becomes an ascus directly. The young-

[4] Van Beijma Thoe Kingma, F. H. Mykologische Untersuchungen—*Penicillium javanicum* nov. spec. Ver. Kon. Akad. Wet. Amsterdam **26**: 16–19. 1929.

[5] **Emmons, C. W.** The development of the ascocarp in two species of *Thielavia*. Bull. Torrey Club **59**: 415–422. 1932.

est asci are found at the outer end of each branch of the ascogenous system. There is no crosier, no conjugate division of nuclei, and no nuclear fusion in the young ascus. There is no reason why any ascogenous cell should not be transformed directly into an ascus. Under the unfavorable conditions for continued growth such as probably existed in Brefeld's cultures of *Penicillium*, excluded from air as they were, the ascogenous cells might very well have become asci directly on occasion. As to the very fine system of branches found in the interior of the ascocarp of *P. Brefeldianum* it is certain that asci can arise from the ends of their branches as noted previously (FIG. 2, i). One finds mature

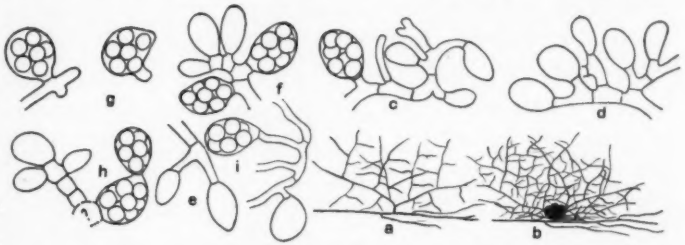

FIG. 2. *Penicillium Brefeldianum.* a, characteristic tree-like branching of the primordial hypha, a sort of subiculum, from which is to arise the perithecium; b, young perithecium, sterile and fertile elements intertwined, the original sterile branched system more highly developed and loosely surrounding the young ascocarp; c–f, various stages in the development of asci from side buds of the cells of the ascogenous hyphae; g, two asci characteristic of those seen in crushed mounts from a mature ascocarp; h, asci in a short chain suggesting that individual ascogenous cells may occasionally be transformed into asci; i, asci produced on the very fine secondary hyphae such as Brefeld considered merely digestive hyphae.

as well as immature asci crowded up against the tissue lining the cavity (PLATE 19, a). The fine hyphae probably take no greater part in the digestion of the stromatic tissue than do the other elements occupying the cavity. The stromatic cells are disorganized by enzymic action rather than by mechanical pressure exerted by hyphal growth. Asci get their nourishing directly by absorption from the surroundings, and not necessarily at all from the supporting ascogenous cell as suggested by Brefeld.

ASCOSPORES

As noted previously the ascospores of *P. Brefeldianum* are spherical to slightly elliptical, and finely echinulate. Van Beijma says that the ascospores of *P. javanicum* are spherical or irregular in form more or less 3-cornered. This difference may be due merely to the action of the mounting fluids used. There is certainly no band running around the spore such as Brefeld showed, and such as characterizes spores of certain species of *Aspergillus*. The fact that when a mature ascospore of *P. Brefeldianum* is germinated the spore wall usually cracks off, remaining in two fragments attached to the swollen spore, would suggest a close relationship to Brefeld's species, and under careful manipulation traces of such valve parts on an ungerminated spore perhaps could be made out even in our strain.

CONCLUDING REMARKS

In *Pleospora* the ascocarp is initiated by the parenchymatous division of some ordinary hyphal cell. There are no ascogonial coils or sex organs. The full grown ascocarp body is merely a differentiated stroma within which certain cells begin to disorganize to make room for the growth of other cells which will give rise to the ascogenous system. The important difference here between the young fruit bodies of *Pleospora* and *Penicillium Brefeldianum* is that in the latter the fertile ascogenous tissue is present from the first, burrowing about in, or enclosed by, the sterile stromatic tissue. Both kinds of tissue are continually increasing in amount as the ascocarp increases in size.

We have in the Plectascales various amounts of sterile protecting tissue in the mature fruit body. Only a very few species of *Penicillium* have been connected with ascocarpic stages. The perithecia of members of the *P. luteum* group (*P. Wortmanni*, *P. aureum*, *P. Sacchari* and *P. Petchii*) all have the most delicate surrounding periderm consisting principally of a loose weft of interlacing hyphal branches. Such a covering might well be represented in *P. javanicum* and *P. Brefeldianum* by the tangle of branches which supports and surrounds, but is not an integral part of, the ascocarp. *P. Wortmanni* in its simplest races is merely a good *Gymnoascus*. The perithecia of *P. bacillosporum*

and of *P. spiculisporum*, on the other hand, have a well organized wall of compacted hyphal branches but the perithecial cavity is apparently formed entirely as the result of excessive peripheral growth of the hyphae surrounding the ascogenous elements. The perithecium of *P. avellaneum* is bounded by a peridial wall consisting of a single layer of highly differentiated cells. A third type of perithecium and still more advanced, is found in *P. Brefeldianum*, *P. javanicum* and *P. glaucum* of Brefeld, where the central cavity is hollowed out of a rather solid pseudoparenchyma stromatic tissue through digestion brought on in connection with ascogenous growth. The older the fruit body the larger the cavity and the thinner the stromatic wall layers (PLATE 19, b). It starts out to be of the *Gymnoascus* and *P. Wortmannii* type as is shown by the tangle of hyphal branches loosely gathered around the ascocarp, and which in Brefeld's form and the van Beijma race, is quite compact. We get from this some idea of how the Gymnoascaceae (if such there are distinct from the *Penicillium luteum* sort) evolved into species like *P. Brefeldianum*, *P. javanicum* and *P. glaucum*. From this to *Microascus* (*Scopulariopsis*) with an ostiole is not a big step if one goes by way of *Thielavia Sepedonium*, where the conidial stage shows a *Penicillium* ancestry just as *Scopulariopsis* does.

With the discovery by Sopp,[6] Curzi,[7] and Emmons and Dodge,[8] that certain species of *Scopulariopsis* have a *Microascus* perithecial stage, the theory that such species are not true *Penicillia*, finds further support. The ascocarps are dark colored or carbonaceous and distinctly ostiolate. Ascospores are discharged in long cirrhi. The growth of the ascogenous hyphae outward and downward from a centrally placed ascogonium shows, however, a relationship to *Penicillium* and the other "Plectascales."

It is necessary in identifying a species of *Penicillium* from its conidial stage to grow the fungus on some standard medium where the color changes can be followed, and certain peculiarities

[6] **Sopp, O. J.** Monographie der Pilzgruppe *Penicillium* mit besonderer Berücksichtigung der in Norwegen gefundenen Arten. Skr. Vid-Selsk. Kristiania **1912**: Mat.-Nat. Kl. **1**: 1–208. 1912.

[7] **Curzi, M.** Una nuova specie di *Microascus*. Boll. Staz. Pat. Veg. **10**: 302–310. 1931.

[8] **Emmons, C. W. & Dodge, B. O.** The ascocarpic stage of species of *Scopulariopsis*. Mycologia **23**: 313–331. 1931.

in the manner of growth of the mycelial hyphae and the conidial system can be studied carefully. We may therefore conclude that, so far as Brefeld's account of the conidial stage goes, his *P. glaucum* and our *P. Brefeldianum* may or may not be identical. But when we compare the diagnostic features of the ascocarpic stage we see first that his ascocarps were much larger 800–1000 μ, while ours seldom exceed 200 μ. His ascospores were possibly a little larger, 3 $\times$ 5 μ, and showed two valve parts like an *Aspergillus* ascospore, while ours are 2.5–3 $\times$ 3–4 μ, mostly spherical, finely echinulate, but have no other wall marking that can be made out readily. This one feature alone would serve to distinguish the two species. The methods of the origin of the asci in the two species are also different, but when one reads Brefeld's account carefully and everything is considered and due allowance made, perhaps not so radically different. As a rule asci of *P. Brefeldianum* are borne as side or terminal buds from the ascogenous cells. Brefeld figures a single ascus on a short stalk in his plate 6, fig. 36, a. He would, however, say that such a cell would ordinarily bud out to form another ascus. When asci are occasionally formed in a short chain their contents are never in open connection as figured by Brefeld in his plate 6, figs. 36, 38, 39. It is the individual cells of a fertile hypha that then become asci directly, and the adjacent asci must obviously be already separated by a septum. Brefeld does not say that the septum is put in between two asci after they are differentiated as such, and have ascospores. One ascus does not bud out like a yeast cell to form another after spores are delimited.

Knowledge of ascus formation in other groups was not extensive in Brefeld's time and his idea of ascus formation in *Penicillium* was a matter of interpretation of what he saw in his mounts, which, he continually reminds us, showed such a tangle that he was forced to write some of the story without being quite sure of what was going on. This will in no way detract from the great value of his many accurate observations and his beautiful drawings which will stand for all time as models and be an inspiration to students of the fungi. It is interesting to learn that there are certain species in which most of the asci are formed in chains, while in other species the asci are commonly formed on short stalks or buds much as they are in *Thielavia Sepedonium*.

SUMMARY

The conidial stage of *Penicillium Brefeldianum* belongs to the monoverticillate group. The species is homothallic, producing its ascocarps readily in culture. Fertile as well as sterile elements of the fruit body arise in the fork of a special tree-like hyphal complex, which is the first set of elements concerned with ascocarp formation. Ascogonia, paired spirally coiled bodies, or sex organs, if any such develop, must come second. The mature ascocarp remains attached to this primary network and through its stalk derives nourishment from the mycelial hyphae in the medium. The young perithecium is composed largely of a stromatic pseudoparenchyma tissue showing little differentiation. At the center ascogenous elements increase in extent at the expense of the stroma. Under good culture conditions the asci mature within two weeks after sowing the spores. There is no hard sand-like sclerotial resting stage.

The ascus usually arises as a side or terminal bud from a cell of an ascogenous branch. A septum cuts off the ascus from the stalk, or the cell from which it arises as a bud. Rarely are asci of *P. Brefeldianum* and *P. javanicum* found in a chain. This has been found to be more common in certain other species. In this case the fertile cells instead of sending out side buds or branches, would become transformed into asci directly. The idea that the asci are formed as swellings along the course of a non-septate hypha, the adjacent asci being connected by isthmuses and not separated until later, if at all, by cross walls, is a misconception due largely to a misunderstanding of Brefeld's account. His figures of ascus formation are perhaps misleading in that they frequently show asci in open connection. Such a method would certainly be anomalous among the Ascomycetes, but in the absence of crosiers there is no reason why adjacent cells of the ascogenous hyphae may not become asci directly as frequently occurs in certain species.

This study of *P. javanicum*, and *P. Brefeldianum* as represented by our cultures Ed 24, proves that there is a group of species of *Penicillium* having a perithecium comparable to that of *P. glaucum* of Brefeld. These ascocarps, while lacking the dark-colored carbonaceous wall of *Thielavia*, are nevertheless much the same

in their organization. The perithecia of species belonging to the *P. luteum* group are very different, lacking entirely the so-called "sclerotium" complex, which is merely a stromatic tissue within which fertile elements are continually developing.

THE NEW YORK BOTANICAL GARDEN
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COLUMBIA UNIVERSITY

EXPLANATION OF PLATES

Penicillium Brefeldianum

PLATE 18

a–d. Cultures on four different kinds of agar media; a, corn meal; b, potato-dextrose; c, dextrose; d, Czapek's medium. The numerous small whitish bodies shown especially in picture a are perithecia. Those at the center contained hundreds of mature asci. Cultures all 17 days old. Somewhat reduced in reproduction.

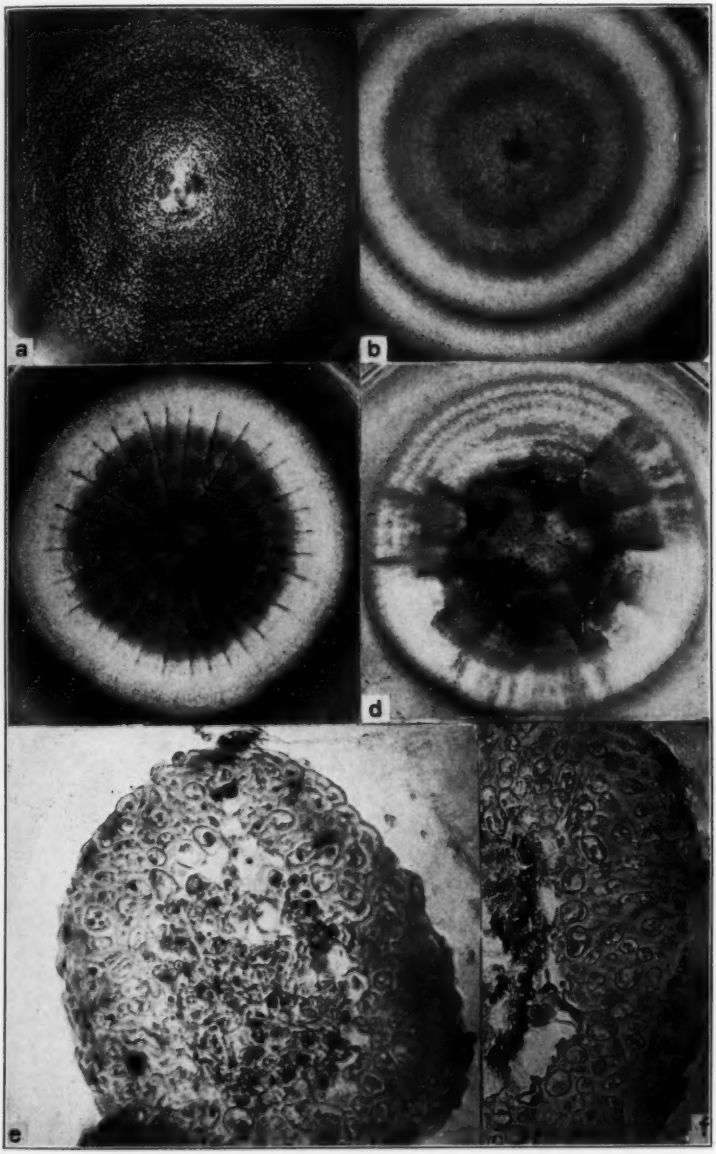
e. Section through the equatorial region of a young ascocarp showing the central mass of ascogenous hyphae and young asci.

f. Portion of a section of a young ascocarp showing the nature of the pseudoparenchymatous sterile stromatic and peridial wall tissues.

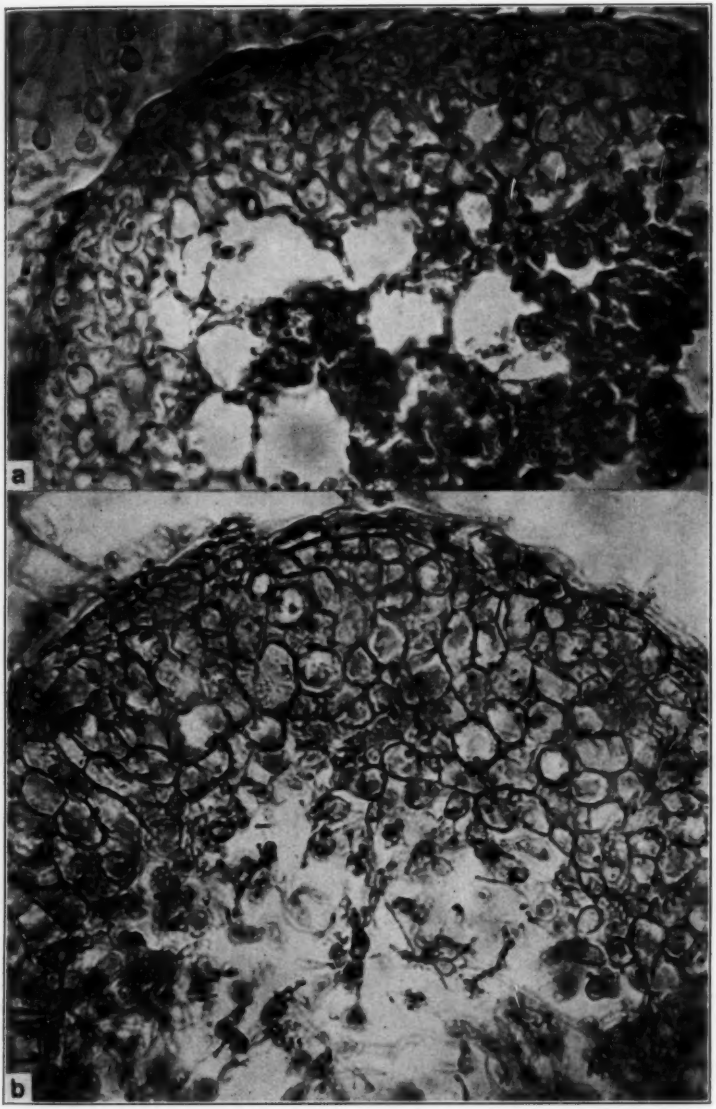
PLATE 19

a. Part of section of a mature perithecium showing disorganization of sterile tissue and mature asci.

f. Perithecium from same culture as a. No suggestion of sclerotize tissue; cavity well formed. Compare with plate 18, f, as to the difference in the appearance of the cells of the sterile tissue.



10 PENICILLIUM BREFELDIANUM



PENICILLIUM BREFELDIANUM

TREMELLA GANGLIFORMIS, A NEW AND UNIQUE TREMELLACEOUS FUNGUS[1]

DAVID H. LINDER

(WITH 1 TEXT FIGURE)

While collecting fungi in Missouri during the early springs of 1930 and 1931, the writer encountered on decaying wood a fungus which because of its small size and its gelatinous texture appeared to be a member of the Tuberculariaceae of the Fungi Imperfecti. When examined under the microscope, however, the specimen proved to be not an imperfect fungus, but a member of the Tremellaceae.

The fungus grows on the under side of very wet decaying wood of a prostrate elm tree. With an excess of water, the fungus appeared to form colonies of many white pustules, but as soon as the excess of water evaporates, the pustules, under a hand lens, can be seen to be connected by strands, of various shapes and sizes, which run over the surface of the substratum, and do not as in most other members of this group, break through the surface of the substratum in more or less irregular lines that follow the grain of the wood. Some of the connecting strands are almost flat and of equal width throughout their length, but the majority are more or less rounded and with occasional swellings and hence resemble the ganglia of certain of the invertebrates. However, the resemblance ends at this point since instead of being relatively straight and unbranched, the chain of gelatinous pustules may turn nearly at right angles, and also frequently is branched, in which case the diverging strands appear to have their origin from the base of a swollen portion of the strand or from a pustule (FIG. 1). If the thinnest strands be studied under the low power of a compound microscope, it can be seen that there is a general parallel arrangement of the elements, which however, because of their small diameter, can not

[1] Contribution from the Cryptogamic Laboratories at Harvard University no. 115.

be clearly discerned. In the thicker strands the parallel arrangement is obscured by the hyphae which have arisen by branching from the horizontal elements.

When a pustule is mounted in lactophenol-cotton blue and carefully crushed under a cover glass, it is difficult to find the hyphal elements of the original strand since they appear to lose the greater part of the protoplasmic content by the time that the pustules are well formed. There are, however, a few hyphae

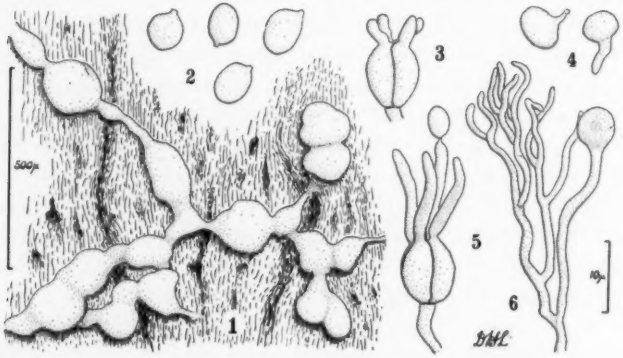


FIG. 1. 1. Habit drawing of the fruiting bodies to show the strands connecting the pustules. 2. Basidiospores. 3. Young basidium with developing sterigmata. 4. Germinating basidiospores. 5. Mature basidium. 6. Very young basidium with a single large fusion nucleus, and the paraphysoid sterile hairs.—All drawings made with a camera lucida, figure 1 to the scale shown at the left, the remainder to the scale at the right.

which run parallel with the substratum and from these arise the slender (less than 1 μ in diameter), much branched and vacuolate hyphae that do not stain readily. These hyphae, rather closely associated, are imbedded in a gelatinous matrix and make up the bulk of the fruiting bodies. Just below the surface of the fruiting body, certain of the branches become enlarged towards their apices and are crowded with protoplasm which has a strong affinity for the cotton blue stain. These branches continue to elongate and enlarge terminally and finally at the apices they bulge out to form the young, subglobose basidia (FIG. 6) in which, judging by the size of the nucleus in each one, nuclear fusion takes place not long after the subglobose bodies have become well

rounded. According to the results of Dangeard's [2] studies on *Tremella mesenterica* the nucleus divides shortly after fusion and the basidium becomes longitudinally divided into four cells, each of which then produces a relatively stout and rather elongate sterigma upon which a spore is produced. A similar procedure is followed by this species, since although the material is not satisfactory for cytological study, the nuclei, conspicuous before division, later become very much smaller and then are readily obscured by the oil drops that are formed in each cell. The basidiospores (FIGS. 2, 4) are subspherical or ovoid and with an oblique apiculus. They germinate (FIG. 4) by sending forth a single short germ-tube at the end of which minute bacteria-like ovoid sporidia are produced.

A careful examination of the hyphae from which the basidia arise makes it evident that all of the branches do not develop into reproductive structures. Instead, many of them continue to elongate and to divide subdichotomously until they project beyond the level of the basidia. These branches, vacuolate and not readily stained, appear to be morphologically identical with the conidia bearing branches described by Dangeard (*l.c.*), but in no instance has the writer been able to find conidia or any evidence of their formation. Since such is the case, it would appear that they are the sterile ends of the hyphae that make up the bulk of the fruiting body, and like them, they are capable of secreting the same mucilaginous substance that forms the matrix of the rest of the pustule. Possibly these sterile ends may be called paraphyses, but if so, in this instance, it would appear that their function is one of secretion and hence they serve to protect the basidia from desiccation. At the same time one should not overlook the possibility that they are conidiophores which, during the evolution of the species, have lost their capacity to produce conidia.

Determination of the taxonomic position of this somewhat unusual species, because of its shape and repent nature, at first offered some difficulties. By using one key to the genera of the Tremellaceae, because of the unilateral hymenium, this species would appear to belong in the genus *Exidia*, whereas in another

[2] Dangeard, P. A. Le Botaniste **4**: 119–181, *figs. 6–7*. 1895.

key, the species, on account of the shape of the spores, would fall into *Tremella*. Furthermore, if the color of the fructification is emphasized as is true in still other keys, this species would be without any apparent connection. A review of the literature in an endeavor to clarify the somewhat confused ideas as to what characters should be used to separate the genera, indicates that such gross morphological characters as color and shape of the fruiting bodies do not serve as well as do the microscopical characters such as the structure of the tissue, the shape of the spores and of the sporidia. According to these criteria, and apparently in agreement with the ideas of most mycologists, the genus *Tremella* is characterized by possessing a rather uniform and gelatinous context, subspherical to ovoid basidiospores, and ovoid, not horse-shoe-shaped, sporidia. The present species agrees in all details with this conception and hence is placed in *Tremella*, but in this genus few of the species are white, all are larger, and not one of them is recorded as producing fruiting bodies of the same striking yet characteristic form. The fungus is therefore deemed worthy of recognition as a new species for which the name *Tremella gangliformis* is proposed.

Tremella gangliformis sp. nov.

Fructificationes resupinatae, gangliformes raro pulvinatae, gelatinosae, albidae deinde cremeae, 0.2–2 mm. longae, 64–144 μ latae; contexto hyalino; basidiis ovoideis, longitudinaliter cruciate septatis, 6.5–7.5 $\times$ 8.5–12 μ; sterigmatibus quater, 1.5–2 $\times$ 36–40 μ; sporis hyalinis, levibus, subglobosis vel ovoideis obliquiterque apiculatis, 4–5 $\times$ 5.5–6 μ.

Fructifications resupinate, gangliform, rarely pulvinate, gelatinous, white drying to cream color, 0.2–2 mm. long, 64–144 μ wide; context hyaline, the hyphae slender, less than 1 μ in diameter, much branched, without clamp connections, imbedded in a gelatinous matrix; basidia when young subglobose, later ovoid and longitudinally cruciate-septate, 6.5–7.5 $\times$ 8.5–12 μ, bearing four sterigmata, 1.5–2 $\times$ 36–40 μ; basidiospores hyaline, even, subglobose to ovoid and obliquely apiculate, 4–5 $\times$ 5.5–6 μ.

On saturated decaying elm wood, near Fenton, Missouri, March 23, 1931, *Linder*. TYPE, in Farlow Herbarium, Harvard University.

OBSERVATIONS ON EPIDERMOPHYTON RUBRUM OR TRICHOPHYTON PURPUREUM[1]

E. MUSKATBLIT

(WITH 3 TEXT FIGURES)

Epidermophyton rubrum belongs to the pathogenic fungi which are rather common in the United States. In Weidman's (**1**) statistics of American species obtained from 272 cases of epidermophytosis and onychomycosis, *Epidermophyton rubrum* was the third organism in order of frequency (17 cases). *Epidermophyton* (or *Trichophyton*) *interdigitale* was the most frequent (140 cases) followed by *Epidermophyton cruris* (54 cases). In our clinic during a period of twenty-three months cultures of various fungi imperfecti were grown from 109 cases of fungous infection of the glabrous skin and nails. As in Weidman's series, *Epidermophyton rubrum* was third in frequency (17 cases), *Epidermophyton interdigitale* being first (55 cases) and *Microsporon* second in order of frequency (21 cases).

Considerable variations in gross as well as microscopic morphology of cultures make the identification of *Epidermophyton rubrum* at times rather difficult. The following brief review of literature makes this point obvious. Castellani (**2**) first described this fungus in 1910 and called it *Epidermophyton rubrum*. Cultures on glucose and maltose agar isolated from cases of *Tinea cruris* were red and showed either a central knob or a crateriform appearance.

Almost simultaneously Bang (**3**) cultivated from lesions of the glabrous skin a species which he called *Trichophyton purpureum*. On glucose medium its colonies were white and downy, with elevated centre, around which there developed later a powdery zone with radial folds. The deep layer of the culture gradually assumed a red color visible only on transillumination or on the

[1] From the Department of Dermatology and Syphilology, University & Bellevue Hospital Medical College, Service of Dr. Howard Fox.

back of the colony. On conservation medium the culture grew slowly, and was covered with a short grayish white duvet, red pigmentation being absent even in old cultures.

In spite of considerable differences described by Castellani and Bang, Sabouraud (**4**) in 1911 expressed the opinion that *Epidermophyton rubrum* of Castellani and *Trichophyton purpureum* of Bang were identical organisms.

Priestley (**5**) in 1917 isolated from erythemato-squamous lesions of the glabrous skin a fungus which he called *Trichophyton rubidum*. On glucose medium its cultures were creamy-white with a short duvet but the medium under and around the colony assumed later a deep red color.

Hodges (**6**) in 1921 described cultures grown from cases of onychomycosis which he provisionally called *Trichophyton* "A" and *Trichophyton* "B." Both produced white and downy colonies. *Trichophyton* "A" developed later a pink color on the surface and purplish red on the back. *Trichophyton* "B" in primary plants became yellowish at places but in subcultures closely resembled *Trichophyton* "A" showing pinkish down on the surface and "purple color from the back." In the supplementary note to his paper Hodges admitted that *Trichophyton* "A" and probably *Trichophyton* "B" were identical with *Epidermophyton rubrum* Castellani and *Trichophyton purpureum* Bang.

Ota (**7**) in 1922 thought that a series of his cultures belonged to the same species although they varied greatly in their morphology. Some were white, others grayish, yellowish, brownish, lilac and red, mostly however without any red pigmentation. Their surface was downy or powdery, sometimes with radial furrows, central knob or irregular depressions and elevations.

Takahashi (**8**) thought that there were two varieties of *Trichophyton purpureum* Bang, one of them producing red pigment.

Bruhns and Alexander (**9**) admitted that white cultures described by Ota and some other Japanese authors as strains of *Epidermophyton rubrum* were possibly pleomorphically degenerated strains of primary red colonies.

Karrenberg (**10**) pointed out that the cultures of *Epidermophyton rubrum* varied greatly in their shape, color, depth of the growth, aspect of the surface, etc. He found it necessary to form

a group of fungi under the name *E. rubrum* including the previously mentioned species of Castellani, Bang and Priestley and also *E. Perneti* Castellani, *E. salmoneum* de Mello and *E. lanoroseum* McCarthy.

Authors disagree also on the question of pleomorphic degeneration. While Bang stated definitely that his cultures did not undergo pleomorphism, Priestley wrote that pleomorphic degeneration not only appeared early but was marked.

Some discrepancies can be found in the description of microscopic morphology of cultures. Castellani found in drop cultures only chlamydospores and a few hyphae with lateral spores. Bang and Ota saw numerous simple hyphae with lateral spores and also branched hyphae with grape-like clusters of spores and multilocular fusiform spores. Priestley described the same picture as Bang but in his cultures fusiform spores were only occasionally present and were not well developed. Hodges in his *Trichophyton* "A" found the same forms of sporulation as Bang and in *Trichophyton* "B" found only a few simple unbranched hyphae with lateral spores and fuseaux.

This brief review of literature shows clearly that the data about *Epidermophyton rubrum* are rather confusing. One gets an impression that two main types of cultures are included in this species. One is red with little or no duvet and with a central knob or crateriform or irregular surface—type *Epidermophyton rubrum*. The other is at first white and downy but later develops red pigmentation in its deep layers—type *Trichophyton purpureum* Bang. Different colonies of this fungus were usually cultivated from different patients. Hodges alone described a remarkable case where two different cultures grew from the same patient. On maltose medium one was downy with central pink area and white periphery, the other was almost smooth with scant purplish duvet. Both cultures, however, were purplish red from the back. On peptone agar the first culture showed downy, pinkish, elevated centre surrounded by a flat smooth area. The second was orange colored with irregularly convoluted centre surrounded by a wide zone made up of elevated sectors divided by radial furrows. Microscopically both types differed in the number of fuseaux, which were more numerous in the downy type. My

observations have a direct relation to this case of Hodges and I believe it would be of interest to report two cases from our clinic, in each of which two entirely different cultures were isolated simultaneously in primary plants. One type was red and cerebriform approaching *Epidermophyton rubrum* Castellani, the other was white and downy with red pigmentation of the basis, fully corresponding to *Trichophyton purpureum* Bang.

DESCRIPTION OF CASES

Case 1. J. M., male 27, negro (FIG. 1) showed on the abdomen and lower extremities numerous erythemato-squamous patches of various size and shape with well defined border. The largest

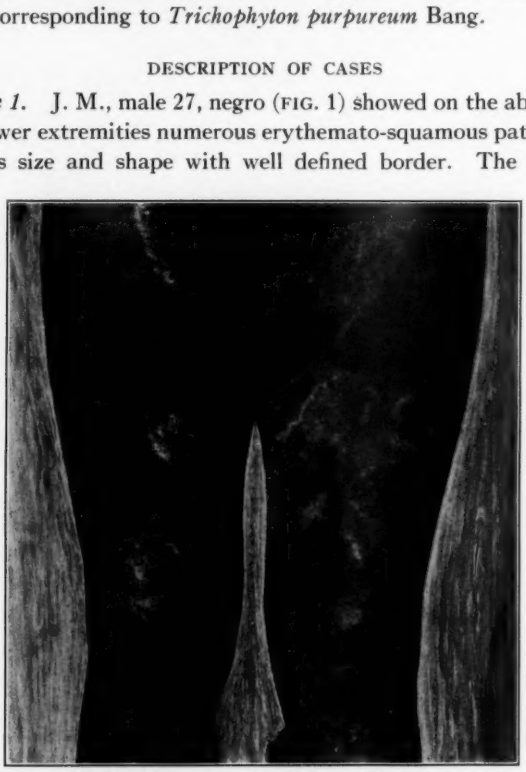


FIG. 1. *Epidermophyton rubrum.* Clinical aspects. Case 1. J. M. (See text.)

lesions occupied the upper and inner aspect of both thigh, the entire picture giving the impression of a generalized tinea cruris. Microscopic examination of scrapings in potassium hydroxide preparation revealed typical mycelia which were long, wavy,

septate and branched. Scales were planted in several tubes with Sabouraud's glucose-peptone-agar medium and gave cultures of two types. Some were red, smooth and cerebriform, others were white and downy. They differed so strikingly from the beginning of their growth that we thought of a mixed infection with two different species.

Case 2. R. L., male, 27, white, presented an eruption of the same type as in case 1, but much more generalized on the trunk, upper and lower extremities, including the palms and soles. All finger nails and all but two toe nails showed typical onychomycosis. Toe webs of both feet were macerated or hyperkeratotic. Microscopic examination of scales taken separately from the trunk, palms, soles, fingernails, toenails and toe webs showed mycelial filaments and chains of spores in all preparations. Cultures were positive from only two locations. White downy colonies grew from the toe web scrapings. Two different cultures, one red and cerebriform, the other white and downy, grew from scrapings taken from the trunk. Further study has shown that white downy cultures from both locations were identical. We have therefore in this case two types of colonies which were similar to those isolated from the first case and require but one description.

A cerebriform culture on glucose-peptone-agar (FIG. 2) started as a knob with smooth but irregular surface and of waxy yellowish color. Pigmentation developed gradually at first in the centre, later also on the periphery. The color was at first lilac and later became deep red. A fully developed culture one month old was 5 cm. in diameter. The centre was considerably elevated. The surface was powdery and made up of numerous cerebriform folds separated by deep furrows. The latter assumed on the periphery a radial disposition with slightly elevated sectors between them. Near the border the culture was flat and even. The color was deep red, lighter on the periphery. The border was sharp, of irregular outline and almost colorless. The surrounding medium at the end of the third month showed a red color particularly noticeable on transillumination. Pleomorphic degeneration developed as a rule and covered the culture with a white dense duvet. The same culture on peptone agar (FIG. 2)

had a similar morphology and was yellowish but at times the centre had a slightly lilac color. Drop cultures showed mycelia, frequently tortuous, thick and irregular with spindle shaped and knoblike terminal swellings, numerous chlamydospores and large multilocular fusiform spores with blunt ends and smooth thin walls. These spindles were the most prominent type of sporulation. Hyphae with lateral spores were present in small numbers in a few cultures. The powder taken from the surface of the culture consisted almost entirely of spindles and chlamydospores. Many mycelial hyphae and chlamydospores contained red pigment in the form of granules and large globules. A downy culture showed quite different gross as well as microscopic morphology. It started on glucose-peptone-agar (FIG. 3) as a white fluffy point and on the fifth week was 5 cm. in diameter. The white downy and raised centre was surrounded by a flat powdery zone with radial furrows and of a pink color more distinct on transillumination. The surrounding medium also showed a red color later.

Pleomorphic degeneration covered the culture with a dense white non-characteristic down.

The same culture on peptone agar (FIG. 3) was again white and downy with a central knob, radial furrows and slightly elevated sectors between them. The powdery pink zone was absent. Drop cultures of the downy colony differed from those of the cerebriform one. Mycelia were straight and much more regular, and some hyphae also contained granules of red pigment. Chlamydospores were much less numerous and multilocular fuseaux were few in number and poorly developed. On the contrary both simple and branched conidiophores with lateral spores and grapelike masses of spores were very abundant. Some hyphae showed the phenomenon of absorption leaving rows of conidia. The powder taken from the surface of the culture consisted almost entirely of small pear-shaped conidia.

SUMMARY AND CONCLUSION

Two different types of cultures were isolated from each of two patients with lesions of the glabrous skin. These two types showed from the beginning of their growth as well as in further

development, different gross and microscopic morphology and behaved like two independent species. The cerebriform type coincided in its main features with *Epidermophyton rubrum* Castellani, the downy type corresponding to the descriptions of *Trichophyton purpureum* Bang. The downy culture cannot be considered as pleomorphic degeneration of the primary cerebri-

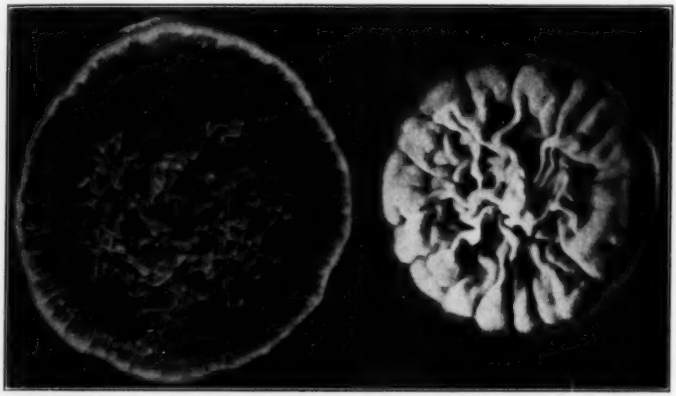


FIG. 2. *Epidermophyton rubrum.* Cerebriform type. Left, glucose-peptone-agar medium. Right, peptone-agar medium.

form one since (1) it developed white and downy as a primary colony directly from planted scales, (2) it showed abundant and characteristic sporulation whereas pleomorphic cultures are sterile or contain few and poorly developed spores, (3) it underwent a pleomorphic degeneration.

Our observations together with the data of the literature allow two explanations.

1. One and the same fungus exists in at least two stable varieties, one red cerebriform with predominance of chlamydospores and multilocular spindle spores—type *Epidermophyton rubrum* Castellani. The other variety white and downy with red pigmentation of the basis and lateral conidia as the main form of sporulation—type *Trichophyton purpureum* Bang.

Both varieties can be isolated in rare instances from the same patient in primary plants.

2. Another explanation might possibly be suggested, that the red cerebriform fungus—*Epidermophyton rubrum* Castellani and white downy one—*Trichophyton purpureum* Bang—are independ-

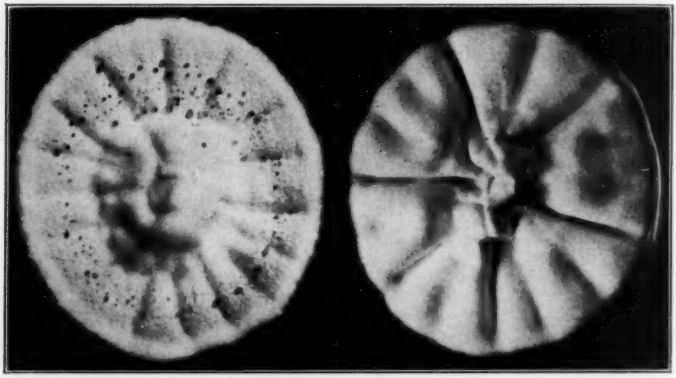


FIG. 3. *Epidermophyton rubrum.* Downy type. Left, glucose-peptone-agar medium. Right, peptone-agar medium.

ent species and may occur in the same patient as a mixed infection with two pathogenic fungi at once.

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VARIATION IN SINGLE SPORE CULTURES OF ASPERGILLUS FISCHERI[1]

H. C. GREENE

(WITH 5 TEXT FIGURES)

In connection with a larger study of the biochemistry of the common fungi it appeared desirable to compare the action of single spore cultures with that of the parent stock cultures. When studying single spore cultures of *Aspergillus Fischeri* Wehmer certain striking macroscopic variations appeared which were investigated and are described in this paper.

Variation within supposedly homogeneous species of microorganisms has of late years been the object of much study, and many conclusions, both coinciding and conflicting, have been drawn therefrom. Within the past decade the special subject of variation in fungi has come to occupy an increasingly important position, and a great deal of information has been accumulated.

Brierley (**8**) has, with considerable success, attempted to classify logically types of variation reported and to correlate them in a connected whole. He divides variations into three principal types:

(1) *Modifications*, non-heritable differences caused by the unequal influence of different conditions, and varying immediately with the conditions.

(2) *Continuous variation*, heritable differences characterized by the gradualness of the change through successive generations.

(3) *Discontinuous variation*, heritable differences characterized by the suddenness of their appearance.

Leonian's recent important paper (**19**) discusses at length the phenomena of variation, and the point is stressed that variation in many fungi is to be regarded as an entirely normal develop-

[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

ment which hitherto has not been taken into account in our scheme of classification.

Many papers, *e.g.*, Arcichovskij (**1**), Schiemann (**21**), Waterman (**23**), Haenicke (**16**), Christensen (**12**), (**13**), Barnes (**2**), (**3**), Johnson (**17**), and Dickson (**15**) have dealt with variation induced or caused by more or less radical alteration of environmental conditions. Such cultural changes are in many cases undoubt-

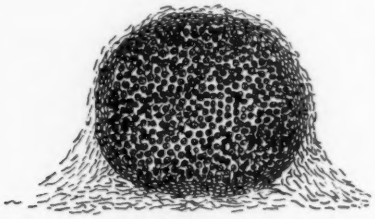

FIG. 1. Section through ordinary perithecium.

edly mere modifications in the sense of Brierley, while others seem to be remarkably permanent. Schiemann's *Aspergillus fuscus*, for example, still retains the distinctive characters Schiemann reported for it in 1912. Chodat (**11**) in discussing his own work with *Aspergillus ochraceus* suggests that the variation is not unrelated to the conditions of the medium in which it appears, but emphasizes that he does not impute to the medium a causal effect in producing variation. He believes that the medium should be considered as a detector which rendered visible a preexisting alteration—a rather fine distinction, to be sure.

As stated, radical changes in cultural conditions are known to produce variations in the characters of various fungi. On the other hand, changes in characters of fungi may take place without any definite modifications of cultural conditions. Many reports, Crabill (**14**), Blakeslee (**4**), Brierley (**7**), Blochwitz (**5**), Stevens (**22**), Leonian (**18**), (**19**), Brown (**9**), Chodat (**11**), Mohendra (**20**), and Brett (**6**) and others, are concerned with variant forms arising principally as sectors or patches of differentiated mycelium in Petri plate colonies. Sector formation is of rather frequent occurrence and any worker is almost certain to encounter it more or less often, depending in some measure on the fungi

under study. The permanence of variants obtained from such sectors is difficult to judge, since the work has been carried out under such widely different conditions. The general impression one gains is that most of the variant forms are relatively unstable, and tend sooner or later to revert to production of characters like those of the cultures from which they were derived.

If we disregard the question of the permanence of variation, the facts still remain that striking and unmistakable cultural changes have been observed by many mycologists in the course of routine stock maintenance, and that such phenomena must be studied if mycological taxonomy is to be placed on something approaching a stable basis.

Brierley (**8**) emphasizes the point that there has been too much undirected work in the past, and that too little has been done in the way of constructive attempts to analyze and to correlate data. He states that he believes that "the investigations of the past few years show clearly the possibility of applying the fundamental concepts, criteria, and terminology of genetics to the last remaining groups, the fungi and bacteria." Dodge has successfully done so in his work on the heterothallic *Neurospora*. There remain, however, many fungi, homothallic and difficultly susceptible to ordinary cytological methods, which present most interesting variations, and which should be studied.

The following account of variations shown by a certain culture of *Aspergillus Fischeri*, for example, while not based upon any exact genetic criteria, nevertheless offers, it is believed, some points which may be of value to workers interested in morphological stability of cultures. With the existent uncertain state of mycological technique in general, the practical aspects of culture maintenance seem to be even more worthy of immediate attention than does the development of a body of genetically exact theory, and indeed it would seem that the latter can only come with vast improvements in empirical technique.

HISTORY AND GROSS MACROSCOPIC APPEARANCE OF THE STOCK CULTURE, *A. Fischeri* 5041

Aspergillus Fischeri Wehmer (FIG. 4, A) is one of the comparatively few Aspergilli producing both conidia and ascospores.

Thom states that the conidial form of *A. Fischeri* is not distinguishable in morphology from *A. fumigatus*. The particular culture of *A. Fischeri* used in this study was received from Charles Thom of the Bureau of Chemistry and Soils, U. S. D. A., as his culture, *A. Fischeri* 5041. The culture was carried by Thom on Czapek's agar, and has been carried in this laboratory since

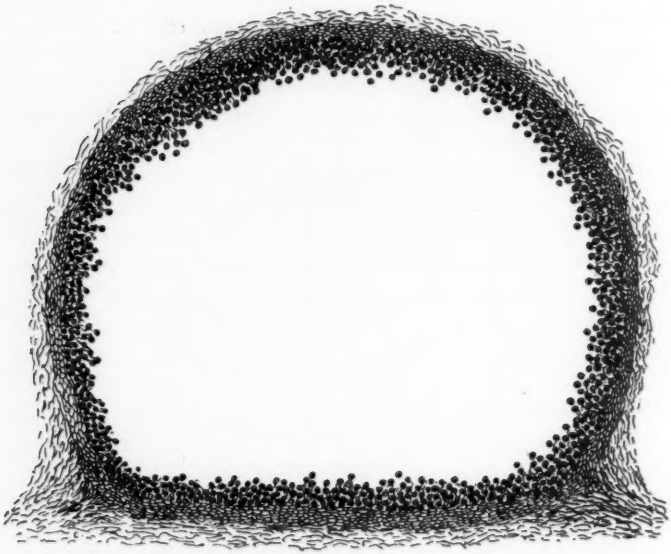

FIG. 2. Section through variant perithecium.

September, 1930, on malt extract agar of the same composition as that used throughout this study. It must be remembered that the following description of gross cultural characters applies necessarily only to the particular culture used, under the special conditions of this study.

When *A. Fischeri* 5041 is transferred to fresh slants or plates of malt extract agar, sterile, white, not pronouncedly aerial mycelium first appears. The white is soon replaced by a light gray-green due to the sparse, but fairly uniform, production of conidia over the whole growth area. This represents the appearance of the culture at about the end of the third day. Shortly

after this the culture begins to show small, whitish flecks, evenly and uniformly distributed throughout. The flecks are immature perithecia. At the end of approximately ten days the white perithecia are mature, that is to say, their content of asci and ascospores is fully developed. The mature perithecia assume such size and become so closely crowded that the rather short conidiophores are no longer apparent, and the whole appears as a practically pure stand of white, globular perithecia (except at the point of inoculation where conidial development is somewhat greater), about ⅓ mm. in diameter, or somewhat less. In the case of a slant, at its uppermost tip, where the agar has dried down, perithecia are not produced, while the gray-green conidia appear in profusion. The characters of the stock have been maintained with constancy, and following mass transfer no significant variation has occurred during the two years that the culture has been carried.

EXPERIMENTAL

Handling of the stock culture and of single spore cultures. All cultures were kept under uniform conditions. They were carried on malt-extract agar of pH 5.0, were kept continuously at 26° C. in a darkened incubator, and were transferred at one month intervals. Many of the cultures have not been kept in stock, but were discarded as they dried up. The malt-extract agar is made up as follows: plain malt-extract (Trommer's Analyzed) 25 g., agar 20 g., distilled water 1000 cc.

Method of isolation of single spore cultures from A. Fischeri. Because of the small size of the hyaline spores, the simple methods adapted to the isolation of large, dark spores, cannot be used here. It is, therefore, essential to employ a method such as gives satisfactory and certain results in the isolation of single bacterial cells. A modified Chambers micro-manipulator was found to be well adapted to the work (**15**a).

In preliminary trials about 50 ascospores were isolated on clear malt-extract agar drops, but under the conditions used, the spores without exception failed to germinate. The expedient of first germinating the spores in malt-extract broth, and isolating germinated spores was resorted to with success. All single spore

cultures studied, whether from ascospores or conidia, were derived from pre-germinated spores. The single germinated spores were deposited on drops of malt-extract agar, on sterile cover slips. The cover slips, with agar drop and spore, were sealed with sterile vaseline to deep hanging drop slides, and were incubated for 48 hours in a darkened incubator. The development of the spores can be followed in detail in the early stages, and the danger of carrying unwittingly contaminated cultures is reduced to a minimum. After the incubation period, cultures were transferred with a small, sterile spatula to ordinary culture tube slants of malt-extract agar and maintained in the usual way.

Isolation of germinated ascospores of A. Fischeri 5041. Ascospores of *A. Fischeri* are characterized by being enclosed between two rather closely fitting valves. The valves, due to the projection of their edges, form an equatorial ridge about the spore, and ascospores are thus, by their distinctive appearance, easily differentiated from the conidia which have no such valves. When the ascospores germinate the valves are forced apart and may, or may not, adhere to the germinated spore. Since a germinated ascospore which has shed its valves does not differ in any well-defined and tangible way from a germinated conidium, it is necessary in isolating germinated ascospores to pick only those which show the two characteristic valves still clinging to the spore.

In the course of this study it became desirable to isolate both germinated ascospores and germinated conidia, and a procedure was adopted with this in view.

Methods for the differentiation of germinated conidia from germinated ascospores in the isolation of single spore cultures. To secure comparable results, it was deemed necessary to isolate both conidia and ascospores from the same slant culture. Since spores were isolated only after germination, and, as mentioned, a germinated conidium cannot with certainty be differentiated from a germinated ascospore which has lost its valves, two practical courses for separation lay open:

1. The isolations of conidia could be made from a 4 or 5 day old, initially ascospore-free culture, whose own ascospores had not yet matured. Such a culture would of necessity be a subculture obtained from a stock slant culture. The mass inoculum, con-

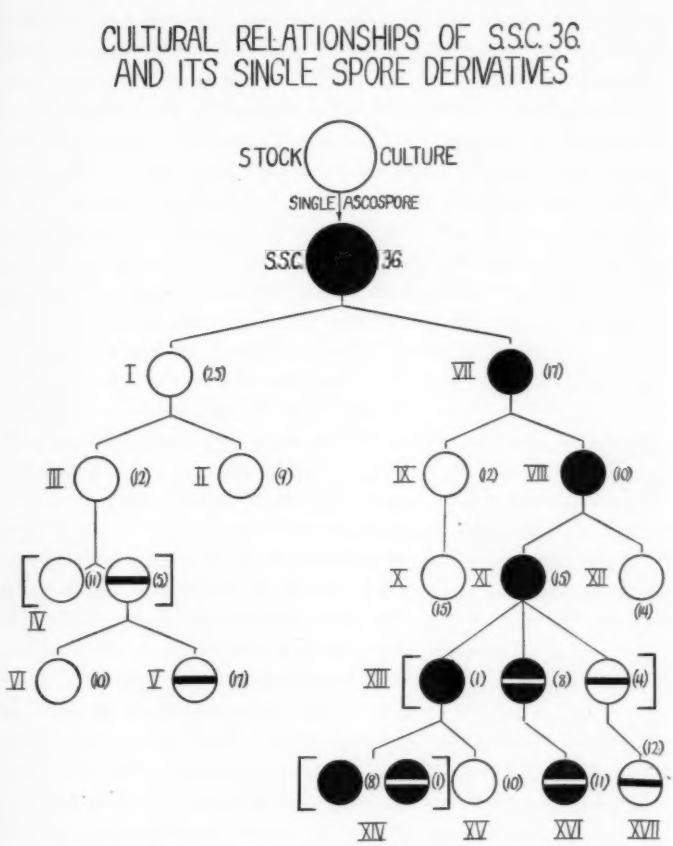

FIG. 3. Roman numerals refer to cultural group numbers (see pp. 128–131). The number of cultures contained in each of the cultural groups indicated by the Roman numerals may be ascertained by reference to the Arabic numerals in parentheses. Unshaded circles indicate cultures resembling the original stock culture, *A. Fischeri* 5041. Completely shaded circles indicate variant cultures resembling S.S.C. 36. Circles with a black central bar indicate cultures showing a slight tendency toward the production of variant characters resembling those of S.S.C. 36. Shaded circles with a white central bar indicate variant cultures showing a tendency toward reversion to the production of characters resembling those of the stock culture.

sisting of a mixture of mycelium, conidia, and ascospores, would be placed as a drop of water suspension on the surface of an upright agar slant. Then, as the mycelium grew toward the upper limit of the slant, and before ascospores matured, a transfer could be made, ascospore-free to another slant, and the conidia for isolation obtained from the latter slant. This procedure was tried out and some single conidium cultures were obtained from the central portions of various slants (primary conidial isolations from S. S. C. 36, in part—see p. 130). It was found, however, that to secure an adequate suspension of conidia it was necessary to so disturb the growing culture as to introduce a serious question as to the comparability of the single ascospore cultures to be obtained later in the growth period. Further than this, assuming that the disturbance of the culture did not of itself influence results, there was the ever-present cultural age factor. That is to say, conidia would be isolated when the culture was 4 days old and ascospores not until 12 days old. This procedure was therefore abandoned.

2. In the ordinary slant of *A. Fischeri* and such of its single spore derivatives as studied (aside from cultures like single spore culture 20), the following obtains when the culture has reached an age of 2 to 3 weeks. There are relatively few conidia in the deeper portions of the slant, while perithecia are produced there in greater or less profusion. Conidia are developed abundantly only at the dried-down uppermost tip of the slant. Perithecia have never been observed in this upper portion. Therefore, the following procedure was adopted and adhered to throughout the study. Cultures were allowed to attain to complete maturity undisturbed, and then conidia from the uppermost portion were transferred to suspensions, the isolation of conidia always shortly preceding that of ascospores. The ascospores are rather firmly held in the semi-mucilaginous, non-ostiolate perithecia, and do not tend to fly about under any conditions. The possibility of the presence of stray ascospores was repeatedly checked by extended microscopic observation, both when isolations were being made and otherwise, and, in cultures handled as described, no ascospores were ever observed among the conidia. Conidia, under the conditions here used, germinate freely within 8

hours. On the other hand, no germinated ascospore with valves adhering, and very many have been observed, has ever been seen within less than 15–16 hours after incubation. Conidia were always picked within 8–10 hours after being placed in the malt-extract broth. Both conidia and ascospores were isolated from slants 2 to 3 weeks old, or in some instances somewhat older.

In all, 448 single spore cultures have been derived, either directly or indirectly, from the stock culture.

Morphological variations in single spore cultures 1–79. The great majority of these single ascospore cultures reproduced, on initial culturing (FIG. 4, b), the characters of the stock culture from which they were derived, namely *A. Fischeri*. Thus, the fungus is definitely homothallic. Certain striking gross variations were manifested in a few of the cultures. These variations may be described as follows: (S.S.C. will hereafter be used to indicate "single spore culture").

S.S.C. 20 (FIG. 4, d). A profuse stand of conidiophores bearing the characteristic gray-green conidia was produced. Perithecia were formed very sparingly and tardily, appearing only about a week after isolation. There was little or no aerial mycelium when the culture was first isolated.

S.S.C. 21. This was similar to S.S.C. 20, but had abundant, whitish aerial mycelium which later turned a gray-green color as a result of production of conidia.

S.S.C. 36 (FIG. 4, c). S.S.C. 36 differed strikingly from the stock culture in forming comparatively few, scattered perithecia, some of them of very great diameter. The average size of a large number of perithecia was about 350 μ with very few under this, but in addition there were also a considerable number of very prominent perithecia, so large that it was not felt that they could be rightfully included in an average. In general, size increased proportionally as the distance apart of the perithecia increased. Hence the large perithecia, standing by themselves, were very conspicuous. The largest perithecium measured was more than 1800 μ in diameter, while five of the ordinary large perithecia, taken at random, measured: 1040 μ, 925 μ, 885 μ, 850 μ, 1000 μ. Comparatively few asci were borne in the large perithecia, contrary to the state of affairs existing in the small perithecia, and

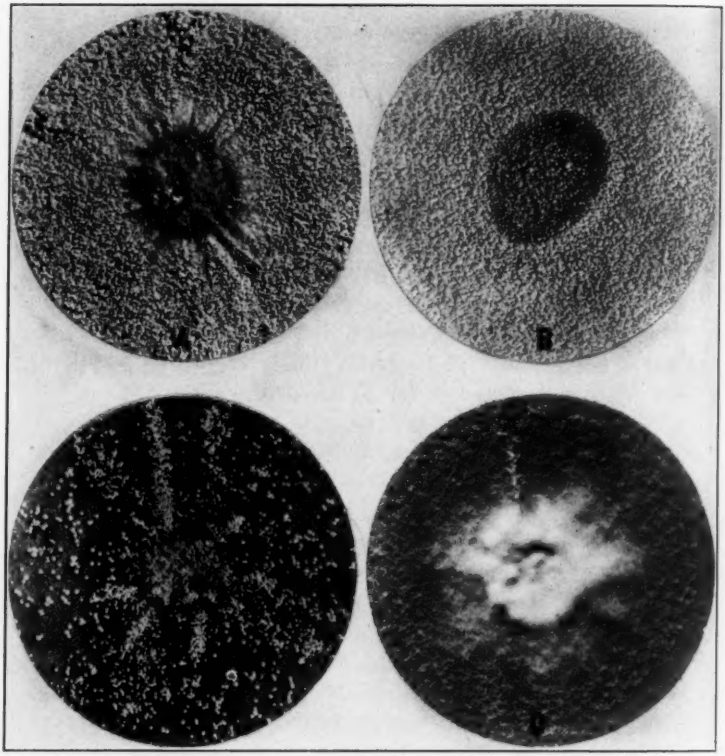

FIG. 4. A, The stock culture, *A. Fischeri* 5041, showing the small, white closely distributed perithecia. (The darker central area is a conidial overgrowth arising at the point of inoculation of the colony); B, S.S.C. 49, a typical single ascospore culture, resembling the stock; C, S.S.C. 36, a variant single ascospore culture, showing the comparatively large, scattered perithecia. (Photograph taken 1 month after original isolation); D, S.S.C. 20, a variant single ascospore culture, showing conidial development, with practical exclusion of perithecial formation. (Photograph taken after 3 months original isolation.)

consequently as the culture aged, the perithecia tended to collapse (see FIGS. 1 AND 2). Ascus and ascospore size remained constant, regardless of perithecial variation.

S.S.C. 38, 51, 52, 59, 74, 78. All these cultures were similar to S.S.C. 20.

S.S.C. 76 (FIG. 5, a, b). This resembled S.S.C. 36, but had even larger perithecia. The average diameter of 50 of them, excluding certain immense individuals as in the case of S.S.C. 36, was about 500 μ. The largest perithecia measured from 2000–3000 μ in diameter.

Morphological variations appearing in single spore cultures 80–108. These cultures were derived from single conidia, as opposed to S.S.C. 1–79 of ascospore origin. The variant cultures are as follows: S.S.C. 83, 103, 104, 106.

These were all similar to the single ascospore variant, S.S.C. 20.

S.S.C. 108. This culture introduced a new type of variation consisting in a dense stand of conidiophores and conidia, with perithecia of ordinary size quite regularly and uniformly placed in the conidial mass. It represents a type intermediate between S.S.C. 20 and the stock culture.

When grown on Petri plates, the variant forms, ascospore or conidial in origin, developed uniformly and reproduced with little sign of further variation, such as sectoring.

Study of variant single spore cultures. S.S.C. 36 showed characters differing in such a high degree from those of the stock culture that a rather extended study of this variant culture was undertaken, and many single spore cultures were derived from it. A short summary of the salient characters of the stock and variant is here presented, and is followed by a description of the work carried out with S.S.C. 36 and its single spore derivatives.

1. *A. Fischeri 5041.* The mature stock culture appears as an almost pure white growth fringed, in the upper dried down portion of the agar slant, with a gray-green band of conidia. Close inspection reveals that the white is due to massed, uniformly and closely distributed, globular perithecia, averaging about 250 μ in diameter. There is no wide variation in perithecial size, and the perithecia are rather compactly filled with asci and ascospores. Conidiophores with their conidia are also to be found

among the perithecia, but the conidiophores are so short and the conidia relatively so few, that they are hardly apparent to the naked eye. On a plate the appearance is similar, with conidia often developing rather abundantly at the point of inoculation and at the edges as the agar dries down.

2. *S.S.C. 36.* This culture varies from the stock in producing comparatively few perithecia, practically all of them of a greater diameter than those of the stock culture, and many of them of a very much greater size. The larger perithecia, especially, are decidedly scattered, and as a consequence stand out very prominently. Conidia appear in the same position and under the same conditions as in the stock culture.

Single ascospore cultures from S.S.C. 36 (*see* FIG. 3, *p.* 123) (*and single spore cultures derived from this ascospore group*):

I. 25 single ascospore cultures from S.S.C. 36 produced, *not* the variant form, but developed without exception into cultures indistinguishable from the stock culture *A. Fischeri* 5041 (from which S.S.C. 36 arose as a single ascospore culture).

II. 9 single ascospore cultures, from one of the 25 primary single ascospore cultures of group I, likewise produced the characters of the stock culture.

III. 12 single conidium cultures, from the same culture as the 9 single ascospore cultures of group II, produced the characters of the stock culture.

IV. 16 single conidium cultures, derived in turn from one of the 12 single conidium cultures of group III, showed in 5 cases a slight scattering of the perithecia. Furthermore, the perithecia had a somewhat larger average diameter than those of the stock culture. In other words, a tendency appeared in the direction of manifestation of characters resembling those of S.S.C. 36. The other 11 single conidium cultures of this group had the appearance of the original stock culture.

V. 17 single conidium cultures, from one of the 5 single conidium cultures of group IV, mentioned as showing a tendency to give rise to variant characters resembling those of S.S.C. 36, showed characters in every case practically identical with those of the parent culture. That is to say, no cultures with the characters of the original stock culture, *A. Fischeri* 5041, were ob-

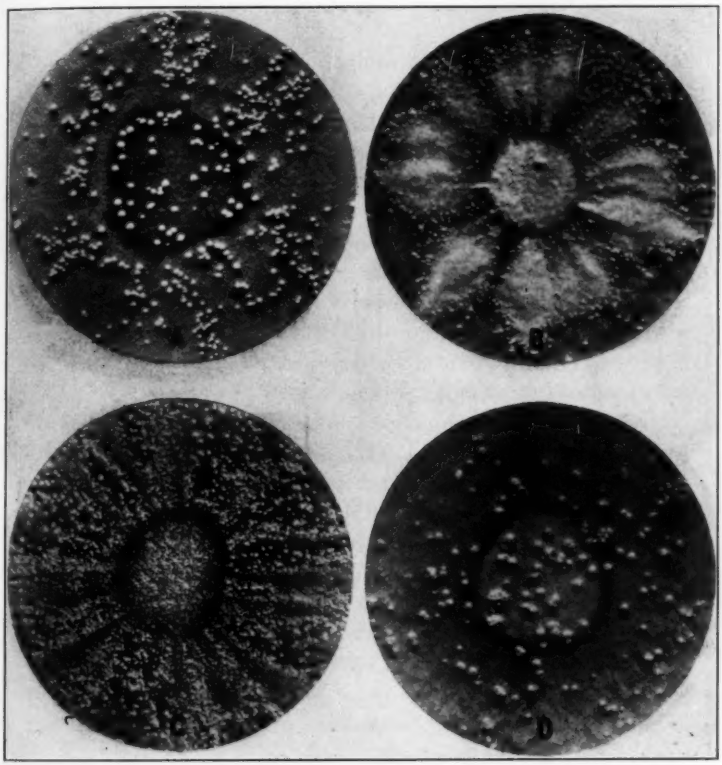

FIG. 5. A, S.S.C. 76, a variant single ascospore culture, even more striking than S.S.C. 36. (Photograph taken shortly after original isolation); B, S.S.C. 76 (Photograph taken after 3½ months of culturing); C, S.S.C. 36 (Photograph taken after 6½ months of culturing, showing the loss of the original variant characters); D, Five-times-removed single conidium derivative from S.S.C. 36, showing the persistence of the variant characters, when carried along through single spore culture series.

tained. However, there was no greater tendency toward characters resembling those of the variant S.S.C. 36 than had appeared in the parent culture.

VI. 10 single ascospore cultures, from the same single conidium culture as the 17 single conidium cultures of group V, produced without exception the characters of the original stock culture, thus differing significantly from the parallel cultures of group V.

This particular cultural line was not further investigated.

Single conidium cultures from S.S.C. 36 (*see* FIG. 3, *p.* 123) (*and single spore cultures derived from this conidium group*):

VII. 17 single conidium cultures from S.S.C. 36 reproduced, without exception, the characters of the variant parent culture. (7 of these cultures, not further used, were obtained by tentative isolation procedure (1) see p. 124.)

VIII. 10 single conidium cultures, from one of the 17 single conidium cultures of group VII, likewise reproduced without exception characters resembling those of the variant S.S.C. 36.

IX. 12 single ascospore cultures, from the same variant conidium culture as the 10 single conidium cultures of group VIII, produced, however, characters like those of the original stock culture, *A. Fischeri* 5041.

X. 15 single conidium cultures, from one of the 12 single ascospore cultures of group IX, showed likewise the characters of the stock culture.

This particular sideline, including groups IX and X, was not further investigated.

XI. 15 single conidium cultures, from one of the 10 variant single conidium cultures of group VIII, reproduced without exception the characters of the variant S.S.C. 36.

XII. 14 single ascospore cultures, from the same variant conidium culture as the 15 single conidium cultures of group XI, showed without exception characters like those of the original stock culture.

XIII. 13 single conidium cultures, from one of the 15 variant single conidium cultures of group XI, showed only one culture which reproduced essentially the characters of the variant S.S.C. 36. Of the other 12 cultures, 8 showed an intermediate tendency

toward production of characters resembling those of the original stock culture, while the other 4 showed a very pronounced tendency in that direction.

XIV. 9 single conidium cultures, from the one single conidium culture of group XIII reproducing the characters of the variant S.S.C. 36, showed likewise the variant characters, with the exception of one culture which showed an intermediate tendency toward characters resembling those of the stock culture.

XV. 10 single ascospore cultures, from the same single conidium culture as the 9 single conidium cultures of group XIV produced, without exception, characters like those of the stock culture, *A. Fischeri* 5041.

XVI. 11 single conidium cultures, from one of the 8 single conidium cultures of group XIII which showed an intermediate tendency toward producing characters like those of the stock, showed in all cases that same tendency, but in no more marked degree.

XVII. 12 single conidium cultures, from one of the 4 single conidium cultures of group XIII which showed a pronounced tendency toward characters like those of the stock culture, showed here the same tendency, but in no more marked degree, in all cases save one. This one exception appeared identical with the stock culture and it is considered possible, but improbable, that a germinated ascospore, minus its valves, was accidentally isolated.

It will have been noted from the study of S.S.C. 36 and its derivatives that all single ascospore cultures, within the large group of single spore cultures derived from the variant S.S.C. 36, produced *not* characters differing from those of the original stock culture, but characters in every case, for all practical purposes, identical. The variations, of whatever type, seem to be transmitted through the conidia, although it must not be forgotten that S.S.C. 36 itself is a single ascospore culture.

Since the variant S.S.C. 76 showed morphological peculiarities similar to those of S.S.C. 36, it was studied along the same lines, to see whether the findings made in the case of the latter culture would hold also for S.S.C. 76.

Single ascospore cultures from S.S.C. 76 (and single spore cultures derived from this ascospore group):

I. 15 single ascospore cultures reproduced the characters of the variant S.S.C. 76. Thus they did not, as did ascospore cultures from S.S.C. 36, produce characters similar to those of the stock culture.

II. 17 single conidium cultures, from one of the 15 single ascospore cultures of group I, reproduced without exception the characters of the variant S.S.C. 76.

III. 8 single ascospore cultures, from the same variant ascospore culture as the 17 single conidium cultures of group II reproduced, in the main, the characters of S.S.C. 76. Only three cultures, however, were as striking as S.S.C. 76, while measurements of perithecia of the others showed them to be about of the order of S.S.C. 36.

Single conidium cultures from S.S.C. 76 (and single spore cultures derived from this conidial group):

IV. 14 single conidium cultures reproduced, without exception, the characters of the variant S.S.C. 76.

V. 13 single conidium cultures, from one of the 14 single conidium cultures of group IV, reproduced in all cases except two the characters of S.S.C. 76, while the two exceptions were, as measurements showed, about of the order of S.S.C. 36.

VI. 11 single ascospore cultures, from the same single conidium culture as the 13 single conidium cultures of group V, reproduced in 5 cases the characters of the variant S.S.C. 76, while the other 6 were somewhat less pronounced, although still very distinctive.

The study of S.S.C. 76 and its derivatives was not carried further. The results show that, whatever its morphological similarity to S.S.C. 36, the resemblance ended there, insofar as ascospore derivatives are concerned.

S.S.C. 20, representative of the second outstanding type of variation from the stock culture, was the source of certain single spore culture groups, as outlined in the succeeding paragraphs.

Single spore cultures from S.S.C. 20:

I. 8 single conidium cultures from S.S.C. 20 reproduced the characters of this variant culture.

II. 9 single ascospore cultures from S.S.C. 20 likewise reproduced the characters of the parent.

At a somewhat later date a number of Petri plate cultures of S.S.C. 20 were made, and numerous wedge-shaped sectors appeared (these had not occurred in earlier plates) which were composed of perithecial stands, like those of the stock culture. It is not considered probable that any of these sectors arose as a result of contamination with spores of the stock culture for the appearance of all plates was homogeneous, and no foreign contamination of any sort was observed, contamination which would be expected had the handling of the plates been so faulty as to allow such wholesale seeding with spores, presumably from the stock culture.

III. 10 single conidium cultures from such a plate reproduced, without exception, the characters of S.S.C. 20.

IV. 9 single ascospores derived from perithecia of a sector showed numerous scattered tufts of white, aerial mycelium, but were otherwise similar to S.S.C. 20.

Cultural characters of single spore cultures upon repeated transfer. Insofar as investigated, the cultural characteristics of the single cell cultures, whether of ascospore or conidial origin, do not, upon the whole, appear to remain fixed. The type which on first culturing showed characters similar to those of the stock culture seems in most cases, but not in all, to change more or less rapidly after three to five transfers. Transfers were made at one month intervals, the cultures being continuously maintained at 26° C. The change is manifested by a more or less sudden loss, in large part, of perithecial production with a predominant gray-green conidial overgrowth becoming the outstanding feature of the culture. The stock culture can be made to assume somewhat of the same appearance simply by growing it at 35-37° C., a thing which seems to favor production of conidia and suppression of perithecia. There has appeared no tendency to revert back from this conidial growth when once it becomes established.

Single spore cultures of the type of S.S.C. 20 (vigorous conidial production upon original isolation) eventually go to the formation of large amounts of sterile mycelium, with conidial production apparently considerably diminished, although this is a difficult matter to judge.

S.S.C. 36 (FIG. 5, c) is not as distinctive as when first isolated

in a large measure resembling the stock culture, but it has not gone over to excessive conidial growth. Such cultures as were derived from S.S.C. 36 and its successive derivatives have, as far as they have been maintained in stock, shown considerably greater constancy in their characters, than single spore cultures derived directly from the stock culture. It should be noted that all single spore cultures except the seven mentioned on page 130 from S.S.C. 36 and its derivatives were obtained from *original* single spore isolation cultures. That is, no single spore cultures were obtained from subcultures of any other single spore cultures, but all were secured directly from the original slants of the cultures in question. Using this system it has proved possible to obtain, five months after the isolation of the original S.S.C. 36, several-times-removed derivatives, which exhibit characters fully as striking as, or more striking than, those of S.S.C. 36 at the time of first isolation (FIG. 5, d), although it has now in a large degree lost those characters.

DISCUSSION

The results obtained with S.S.C. 36 and its single spore derivatives indicate a definite difference in the developmental potentialities of conidia and ascospores, in the case of this particular culture. S.S.C. 76, however, a variant with similar morphological features showed no such differentiation. Apparently, no generalization can be set forth concerning the behavior of single spore cultures derived from variants of this type.

In view of the attempts made to maintain uniformity of environmental conditions, the phenomena described can hardly be explained solely on the basis of changes in such conditions.

An objection may be advanced that, since single conidium cultures were grown from spores taken from the upper, dried-down tips of agar slants, while ascospores came from perithecia in the moist central portion of the culture, here is a difference in environment which might be sufficient to cause significant changes.

Christensen (**12**) studied variation in *Helminthosporium sativum* and found that sectoring occurred only in the thinner agar layer of slanted Petri plates, which layer is, of course, comparable to the tips of slants. It is thus possible that one is merely carry-

ing the variation along by taking conidia from the upper region of the slant, and that under comparable conditions there would really be no difference in the potentialities of conidia and ascospores.

Brown (**9**) worked with *Fusarium* and found that the characters of a parent culture could be most readily perpetuated by taking inocula from the growing edge of a culture. He notes that the proportion of inocula which gave rise to variants increased with the age of the culture and was greater in the central region of the culture than nearer the margin. Thus, starting with a variant culture there might be a possibility that when conidia are taken from the margin of the culture, the cultural characters are perpetuated, whereas when ascospore cultures revert to the type of the stock culture, there occurs what is in effect a further variation.

Mohendra (**20**), using various fungi, found that when, under specified conditions, he employed inocula of different types such as old mycelium, young mycelium, and spores, he was able to demonstrate no variations, even though the fungi were perpetuated in this fashion through a number of generations.

The results of Christensen and Brown would tend to show that environmental factors may possibly have been operative in the course of the present work. It should be noted, however, that S.S.C. 36.36–36.42 which produced the variant type were derived from conidia from the central portions of young, vigorously growing, ascospore-free cultures of S.S.C. 36, while all the other single conidium cultures which gave rise to the variant type were derived from conidia taken from the tips of slants. Further than this, S.S.C. 36 and S.S.C. 76, both of them variant cultures from the stock culture, arose from ascospores taken from the moist central portion of the slant. Also, single conidium cultures derived from single ascospore cultures within the S.S.C. 36 cultural group did not give rise to cultures differing from the parent culture, except for one case where there appeared a slight tendency in that direction (group IV). Since the data of Brown and Christensen are, in a sense, opposed, it is obvious that by shifting their findings about, one can apply them after a fashion to the facts just mentioned, but certainly not with consistency. If one

seeks to explain the results of the present investigation with S.S.C. 36 on the basis of direct environmental influence, it is hard to reconcile the findings obtained with S.S.C. 76 which was manipulated in the same way, although it must be admitted that there is no direct comparison here.

Chaudhuri (**10**) regards the average case of variation as purely a nutritive phenomenon, and states that the vast majority of variants can be made to revert to their original forms by culturing on a suitable type of medium. Most of the cases of variation to which he refers have been manifest in sector formation. The stock culture, *Aspergillus Fischeri* 5041, has been grown on plates on numerous occasions, using the same medium on which the single spore variants arose, and insofar as sector formation is concerned, has only occasionally given rise to narrow sectors characterized by an overgrowth of sterile mycelium, and never to anything remotely resembling the type of variation exhibited in S.S.C. 36 and S.S.C. 76.

If one is inclined to accept the following definition of mutation as quoted by Brierley (**8**), the variations described in this paper certainly cannot be regarded as being of the order of mutations. Thus, mutation is "The result of a change in genotypic constitution occurring independently of normal segregation, crossing-over, or irregular chromosome division; strictly an alteration in the fundamental nature of the germplasm, usually in a single gene" (Jones 1925). It is as well perhaps to avoid the use of "mutation" and to adhere to such terms as "variation" and "saltation," although as Brown (**9**) says, there seems to be little reason for sharing in Brierley's anxiety over the lamentable consequences of the indiscriminate use of "mutation," for after all, every reader of microbiological literature is so accustomed to the use of the word in the sense of ordinary variation, that no particular confusion is likely to be occasioned when "mutation" is loosely employed.

SUMMARY

Using a modified Chambers micromanipulator, 448 single spore cultures were obtained from a stock culture of *Aspergillus Fischeri*, a perithecial form. Certain of these cultures showed striking morphological variations from the stock culture, and this paper is concerned with a study of the variant cultures.

Variant cultures were of two main types: (1) Very large, scattered perithecia were produced, as opposed to the condition in the stock culture where the perithecia are small, and closely and uniformly distributed. (2) Conidia were produced in profusion, while very few perithecia were formed and those only tardily, contrary to the case in the stock culture.

In the case of a certain culture of type (1), cultures derived from it, both from ascospores and conidia, through several single spore generations reproduced the characters of the variant parent. In another instance, however, single ascospore derivatives through a number of single spore generations produced *not* variant cultures, but cultures practically identical morphologically with the original stock culture. Cultures derived from conidia, on the other hand, reproduced the variant type. Single spore cultures from a type (2) variant reproduced the variant characters, whether the cultures were derived from single ascospores or single conidia.

DEPARTMENTS OF AGRICULTURAL BACTERIOLOGY AND AGRICULTURAL CHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN.

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DERMEA AND PEZICULA[1]

FRED J. SEAVER AND JOSEFA VELAZQUEZ

(WITH PLATES 20–23 AND 1 TEXT FIGURE)

The genus *Dermea* was founded by Fries (Syst. Orbis Veg. 343. 1825) and later changed to *Dermatea* by the same author. Fries changed the spelling of the name apparently for etymological reasons, but the original spelling is here retained in accordance with the rules in spite of the fact that the latter is in common use. Although not the first species mentioned *Peziza Cerasi* of Persoon is usually regarded as the type of the genus and probably fittingly so since it is widely distributed, well known, and a species of economic importance.

In 1865 Tulasne established the genus *Peziçula* based on *Peziza carpinea* Persoon, a species common on *Carpinus*. There seems to have been some difference of opinion as to the grounds for the separation of these two genera. Lindau (E. & P. Nat. Pfl. 1[1]: 235. 1897) regards them as synonyms. Saccardo treats the genera as distinct but the characters on which they are separated by him are vague and the basis for separation not clearly defined.

In working over the various species of the two genera the writer has been inclined to throw them together in one genus, since it seems to be impossible to separate them on ascospore characters. However, more detailed study has revealed an apparently definite morphological character on which the genera can be separated if we wish to resort to the conidial stages. To what extent the conidial stage should be used in segregating genera of the Ascomycetes is an open question since the latter are often obscure or entirely wanting. Fortunately, however, there are usually other characters which accompany the differences in conidial characters by which species can be distinguished even though the conidial stage is not actually seen. Such seems

[1] This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates), which was published by the author and issued in December, 1928.

to be the case with *Dermea* and *Pezicula* so far as our observations have gone.

Dermea has a conidial stage, usually accompanying the apothecial, consisting of soft fleshy stromata with irregular cavities in which pynospores are produced. The pycnospores are long, fusiform, and usually curved resembling those of the genus *Fusarium*. Such forms have usually been referred to *Micropera* or *Gelatinosporium*. On the other hand, *Pezicula* has conidia of a very different type, being large and ellipsoid and borne on the outside of a fleshy stroma. The conidial stage of this type has been referred to various form genera, *Gleosporium*, *Myxosporium*, *Discosporium* and *Tuberculariella*. Or the conidia may be produced in well developed pycnidia of somewhat variable form. These are usually referred to *Sphaeronema*.

Fortunately there seems to be some apothecial characters which accompany these conidial differences. The apothecia in *Pezicula* are soft and fleshy and usually light-colored, yellowish or whitish, the hymenium being plane or convex and more or less roughened by the paraphyses which seem to be entirely free. In *Dermea* on the other hand the apothecia are dark-colored and the hymenium dark-brown or blackish and presenting a smooth surface. The paraphyses are bound together by a dark matrix forming a rather definite epithecium. Whether these apothecial differences will hold throughout the genera is not certain since the conidial stages in many of the species are unknown.

The object of the present paper is to present these differences as they appear at the present time hoping that this will stimulate new activity in the collection and study in various species of the two genera. Special search should be made by collectors for the conidial stage.

4. DERMEA Fries, Syst. Orbis Veg. 343. 1825.

Dermatea Fries, Summa Veg. Scand. 362. 1849.

Apothecia occurring singly or more often in cespitose clusters often on a stromatic base, tubercular in form or discoid more rarely scutellate, usually dark-colored, comparatively small, rarely exceeding 2 mm. and usually 1 mm. or less in diameter, coriaceous to subcarbonaceous; asci usually broad-clavate and 8-spored; spores usually comparatively large, occasionally minute,

simple or becoming tardily 1–several-septate, the septation often erratic even in the same species; paraphyses colored and their tips agglutinated into a dark-brown or blackish epithecium.

Type species, *Peziza Cerasi* Pers.

This genus grades rather closely into *Cenangium* but usually has smaller discoid apothecia and larger often septate spores. The pycnospores in the various species which are produced in irregular pycnidial cavities in a fleshy stroma are fusiform, usually curved and septate. The conidial stage so far as observed belongs to the form genus *Micropera* (*Gelatinosporium*).

DERMEA CERASI (Pers.) Schw. Trans. Am. Phil. Soc. II. **4**: 237. 1832.

Peziza Prunastri Pers. Tent. Disp. Fung. 35. 1797.
Peziza Cerasi Pers. Tent. Disp. Fung. 35. 1797.
? Sphaeria dubia Pers. Ic. Pict. Fung. 48. 1806.
Ceratostoma spurium Fries, Obs. Myc. **2**: 338. 1818.
Cenangium Cerasi Fries, Syst. Myc. **2**: 179. 1822.
Cenangium Prunastri Fries, Syst. Myc. **2**: 180. 1822.
Micropera drupacearum Lév. Ann. Sci. Nat. III. **5**: 283. 1846.
Dermatea Cerasi Fries, Summa Veg. Scand. 362. 1849.
Dermatea Prunastri Fries, Summa Veg. Scand. 362. 1849.
Micropera Cerasi Bonord. Abh. Nat. Gesells. Halle **8**: 133. 1864.
Sphaeronema spurium Sacc. Syll. Fung. **3**: 186. 1884.
Tympanis Prunastri Wallr. Fl. Crypt. Germ. **2**: 427.
Tympanis Cerasi Quél. Enchir. Fung. 330. 1886.

Apothecia bursting through the bark in dense cespitose clusters often 1 cm. in length and usually much narrower, the individual apothecia at first club-shaped discoid, with a thick stem-like base, reaching a diameter of 1 mm., about as high as broad, brownish-black; hymenium plane or nearly so, black; asci clavate, reaching a length of 80–100 μ and a diameter of 12 μ, 8-spored; spores irregularly 2-seriate, ellipsoid, becoming 1-septate, straight or slightly curved, 5–7 $\times$ 15–18 μ (rarely as long as 20 μ); paraphyses filiform, the ends forming a brown epithecium.

Often accompanied by the conidial stage, consisting of soft yellowish stromata with pycnothecial cavities or often forming conical pycnidia 1–3 mm. high; pycnospores fusiform-linear, curved, hyaline, 3.5 $\times$ 40–50 μ.

On branches of *Prunus emarginata*, *Prunus pennsylvanica* and other species of *Prunus*.

TYPE LOCALITY: Europe.

DISTRIBUTION: Pennsylvania to Newfoundland; also in Europe.

ILLUSTRATIONS: Phill. Brit. Discom. *pl. 10, f. 66;* Phytologist **12**: 211, *f. 3* (as *Dermatella Prunastri*); ? Pers. Ic. Pict. Fung. *pl. 20, f. 1–2;* Tul. Fung. Carp. **3**: *pl. 19, f. 13–17;* E. & P. Nat. Pfl. 1^1: 237, *f. 179 A–D;* Rab. Krypt.-Fl. 1^3: 242, *f. 1–6*.

EXSICCATI: N. Am. Fungi *40, 989, 2555, 2812;* Fungi Columb. *3118, 4942;* Reliq. Farlow. *113;* Rav. Fungi Am. *2116;* Rav. Fungi Car. *71*.

According to W. J. Dowson (Phytologist **12**: 207. 1913) causing a disease of greengage plum trees in England.

DERMEA BETULAE Rehm in Rab. Krypt.-Fl. 1^3: 1221. 1896.

Gelatinosporium fulvum Peck, Ann. Rep. N. Y. State Mus. **38**: 97. 1885.

Apothecia usually occurring singly erumpent through the bark and becoming prominent, externally yellowish brown, reaching a diameter of 1 mm.; hymenium nearly plane with an upturned margin, black or blackish; asci clavate reaching a length of 90 μ and a diameter of 16–18 μ, 8-spored; spores irregularly 2-seriate narrow-ellipsoid, at first 1-septate, often becoming 3-septate, 5–7 $\times$ 14–20 μ; paraphyses slender, enlarged above, the apices surrounded by a brown matrix which forms a dark epithecium.

Conidial stage, *Micropera*, consisting of an erumpent yellowish stroma, with irregular pycnothecial cavities from which the spores ooze in a gelatinous mass, pycnospored fusiform, curved, 3-septate 3–4 $\times$ 55–75 μ, hyaline.

On branches of *Betula lutea* and *Betula* sp. and *Alnus* sp.

TYPE LOCALITY: Europe.

DISTRIBUTION: New York to Nova Scotia and Michigan; also in Europe.

The writer has examined what is apparently a part of the type material *Gelatinosporium fulvum* and finds that it agrees with the *Micropera* which accompanies this species and is apparently its conidial stage.

Dermea Brenckleana (Sacc.) Seaver, comb. nov.

Patinella Brenckleana Sacc. Mycologia **12**: 203. 1920.

Apothecia gregarious, erumpent-superficial, occurring either singly or in cespitose clusters of several each, brownish-black, becoming subdiscoid, reaching a diameter of 1 mm.; hymenium slightly concave or plane with the margin slightly elevated, brownish-black, a little darker than the outside of the apothecium; asci clavate, reaching a length of 70 μ and a diameter of 11–12 μ, 8-spored; spores 2-seriate, fusoid, slightly curved 3–4 $\times$ 15 μ (septate ?); paraphyses filiform, hyaline.

The conidial stage consists of fleshy stromata in the cavities of which typical pycnospores are produced. The pycnospores are fusiform, curved and reach a diameter of 2–3 μ and a length of 16–18 μ, and are apparently 1-septate.

On *Amelanchier alnifolia*.

TYPE LOCALITY: Whitestone Gully, North Dakota.

DISTRIBUTION: North Dakota and Montana.

The *Micropera* stage of this fungus was found associated with the apothecia in type material obtained from Dr. J. F. Brenckle. Both stages were also found on bark of *Amelanchier alnifolia* from Montana which was collected by Dr. J. R. Weir (*14921*) but apparently not seen by him, since it bore also *Sphaeronema pruinosa* Peck for which it was collected. The latter is the conidial stage of *Pezicula pruinosa* (Peck) Farlow.

PEZICULA Tul. Fung. Carp. **3**: 182. 1865.

Apothecia usually occurring in cespitose clusters on a stromatic base, sessile or with a short thick stem-like base, usually light colored whitish or yellowish, rarely exceeding 1 mm. in diameter, tubercular or discoid, usually soft and fleshy; asci broad-clavate, usually 8-spored; spores ellipsoid, simple or becoming tardily 1–several-septate; paraphyses hyaline or subhyaline and usually free, not agglutinated and not usually forming an epithecium.

Type species, *Peziza carpinea* Pers.

The genus is distinguished from *Dermea* by the light colored fleshy apothecia and the character of the conidial stage. The pycnospores are broad-ellipsoid and borne externally on the surface of the stroma, *Myxosporium*, or in well developed pycnidia, *Sphaeronema*.

PEZICULA CARPINEA (Pers.) Thüm. Fungi Austr. *767*. 1873.

? Tubercularia fasciculata Tode, Fung. Meckl. **1**: 20. 1790.
Peziza carpinea Pers. Syn. Fung. *673*. 1801.

Cycledum Carpini Wallr. Fl. Crypt. Germ. **2**: 512. 1833.

Dermatea carpinea Fries, Summa Veg. Scand. *362*. 1849.

Apothecia thickly gregarious, springing in cespitose clusters from an immersed fleshy stromatic base, the individual apothecia tuberculate or expanded and subdiscoid, with a short stem-like base, often distorted by mutual pressure, reaching a diameter of 1–3 mm., yellowish; hymenium plane or convex, similar in color to the outside of the apothecium; asci clavate, reaching a length of 150–200 μ and a diameter of 15–20 μ, 8-spored; spores ellipsoid, straight or curved, granular within, for a long time simple but often becoming 1–3-septate, 10–12 $\times$ 18–30 μ; paraphyses slender, branched, enlarged above, reaching a diameter of 5 μ, not forming an epithecium.

The conidial stage of this species as pointed out by Tulasne consists of a soft fleshy stroma on the surface of which the pycnospores are produced. Pycnospores broad-ellipsoid 10–12 $\times$ 20–24 μ, each borne on a slender conidiophore which is strongly swollen just below the point of attachment.

On trunks and branches of *Carpinus caroliniana*.

TYPE LOCALITY: Europe.

DISTRIBUTION: Massachusetts to Missouri and Pennsylvania; also in Europe.

ILLUSTRATIONS: ? Tode Fungi Meckl. *pl. 4, f. 32;* Rab. Krypt.-Fl. **1**^{3}: 243, *f. 1–6;* Ann. Sci. Nat. III. **20**: *pl. 16, f. 17, 18.*

EXSICCATI: N. Am. Fungi *67 b* (as *Dermatea carnea*), *3333;* Shear, New York Fungi *93;* Rab.-Winter, Fungi Eu. *3463;* Reliq. Farlow. *134.*

PEZICULA ACERICOLA (Peck) Sacc. Atti. Ist. Veneto VI. **3**: 725. 1885.

? Peziza cinnamomea D.C. in Pers. Myc. Eu. **1**: 268. 1822.

Sphaeronema acerinum Peck, Ann. Rep. N. Y. State Mus. **24**: 86. 1872.

Nodularia acericola Peck, Ann. Rep. N. Y. State Mus. **25**: 98. 1873.

Dermatea carnea Cooke & Ellis, Grevillea **5**: 32. 1876.

Sphaeronema nigripes Ellis, Bull. Torrey Club **6**: 107. 1876.

Tympanis acerina Peck, Ann. Rep. N. Y. State Mus. **31**: 48. 1877.

? Dermatea cinnamomea Phill. Brit. Discom. 342. 1887.

Scleroderris acerina Sacc. Syll. Fung. 8: 599. 1889.

Dermatea acericola Rehm in Rab. Krypt.-Fl. 1^3: 1245. 1896.

Apothecia erumpent in cespitose clusters of 3–8 each, the individuals seldom exceeding 1 mm. in diameter sessile or subsessile, pale-yellow (becoming blackened with age); hymenium plane or slightly convex, the margin rather indistinct, similar in color to the outside of the apothecium, becoming concave with age; asci clavate, reaching a length of 90–130 μ and a diameter of 15–20 μ, 4–8-spored, gradually tapering below into a slender stem-like base; spores irregularly 2-seriate above, ellipsoid, straight or curved, at first simple, often becoming 3–4-septate, 8 $\times$ 24–26 μ, hyaline or subhyaline, occasionally septate; paraphyses slender, enlarged above and often flexuose hyaline or slightly colored with age.

This species is associated with *Sphaeronema acerinum* Peck which appears to be its conidial stage. Pycnidia minute, black with long, bristle-like, translucent ostiola. Pycnospores broad-ellipsoid, 8 $\times$ 20 μ slightly narrowed at the point of attachment with the conidiophore which equals or exceeds the length of the spore and is slightly swollen just below the point of attachment.

On dead branches of *Acer rubrum* and other species of *Acer*.

Type locality: North Elba, New York.

DISTRIBUTION: New York and Pennsylvania to Newfoundland and Ontario.

ILLUSTRATIONS: Grevillea **5**: *pl. 75, f. 9* (as *Dermatea carnea*).

EXSICCATI: Reliq. Farlow. *112, 143 a–b;* Thüm. Myc. Univ. *978;* Rehm, Ascom. *1901;* Ellis, Nova-Caesar. *56* (as *Dermatea carnea*); N. Am. Fungi *67a;* Fungi Columb. *3420.*

In working over recent collections of *Dermatea acericola* the writer found *Sphaeronema acerinum* Peck intimately associated with them and arrived at the conclusion that this represented the conidial stage of *Dermatea acericola*. Later, however, in looking over the literature of the subject it was discovered that *Sphaeronema acerinum* had been listed by Peck and others as the conidial stage of *Tympanis acerina* in which the apothecia are entirely black and quite different in general appearance from *Dermatea acericola*. This prompted a more careful scrutinizing of the two species and it was discovered that *Tympanis acerina* Peck is merely an aged and blackened form of *Dermatea acericola*. Even in material collected by Peck, which is apparently part of

the type of *Tympanis acerina*, a few of the yellow apothecia were found. In later collections the yellow and the black apothecia were also found intimately associated (see Reliq. Farlow. *112*). Furthermore, both supposed species have asci and spores which are in every way identical leaving no doubt that the two species are synonymous. Hence both the writer and Peck were justified in their conclusions that *Sphaeronema acerinum* represents the conidial stage of the two species which are now known to be synonymous.

Pezicula spiculata Seaver, sp. nov.

Apothecia cespitose in rounded or elongated clusters or rarely occurring singly, reaching a diameter of 1 mm. pale yellowish, sessile or tapering into a short stem-like base; hymenium slightly concave or plane not darker than the outside of the apothecium; asci broad-clavate, reaching a length of 120 μ and a diameter of 16–18 μ, 8-spored; spores irregularly 2-seriate, ellipsoid and often slightly curved 8 $\times$ 24–27 μ, becoming 1–3-septate; paraphyses slender slightly enlarged above, the ends free, branched, hyaline, about 2 μ in diameter.

The conidial stage, *Sphaeronema*, accompanies the apothecial both springing from a floccose stroma. The pycnidia are large and spike-like, black but covered with white flakes reaching a length of 1.5 mm., swollen below. The pycnospores are 10 $\times$ 20–24 μ and borne on sporophores equalling or exceeding the length of the spore.

On *Acer* (*spicatum ?*).

Type collected near Ithaca in connection with the summer foray, Aug. 28–Sept. 2, 1931.

So far as the ascigerous stage of this species is concerned it can scarcely be distinguished from *Pezicula acericola*. However, the two are apparently entirely distinct in their conidial stages. The *Sphaeronema* stage very closely resembles *Sphaeronema pruinosa* Peck which occurs on *Amelanchier* and is the conidial stage *Pezicula pruinosa* (Peck) Farlow.

CULTURE DATA

Pezicula acericola.

In order to prove the connection between *Pezicula acericola* (Peck) Saccardo and *Sphaeronema acerinum* Peck, culture work

was undertaken by the junior author of this paper. The work was based on collections made by the senior writer in the latter part of August and early September, 1931, in connection with the fungus foray at Ithaca, New York. Preliminary experiments showed the ascospores of this species to still be in a viable condition.

On April 6, 1932 a single ascus was planted and germination was apparent the following day. On April 14, 1932 four single spore test tube cultures were made on potato agar. On April 26, 1932 four more test tube cultures were made on Lindegren agar and growth was much more luxuriant than on the potato agar. Many greenish bodies formed some with yellowish growth at the tips. On May 15, 1932 a small white powdery looking growth on plate planted April 7, 1932 was examined and conidia were found abundantly which agreed with the conidia found in *Sphaeronema acerinum*. These are at first simple later appear to becoming 1–3-septate or pseudo-septate not apparent in material collected on natural substratum.

While the spores were found in greenish bodies these bodies

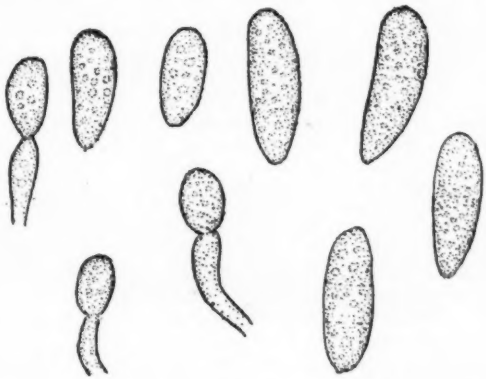


FIG. 1. *Pezicula acericola*. Pycnospores from culture.

did not take on the form of the pycnidia found in nature. They entirely lacked the long filiform ostiole. However, this is probably reaction to substratum since the spores themselves appear to be typical (FIG. 1).

Pezicula spiculata.

This species here designated as new was based on material collected at the same time and the same place as the preceding, *Dermatea acericola*. The pycnidial stages as they occur in nature are so distinct that the writer is inclined to regard them as different species.

In order to demonstrate, if possible, these differences in culture the spores of this species were cultivated simultaneously with *Dermatea acericola* with similar results. However, in neither case did the pycnidia develop sufficiently to show the characteristics that appear in nature.

Unfortunately these experiments were cut short owing to the fact that the junior author returned to Porto Rico early in June. More cultural work should be carried on in order to demonstrate the points brought out above.

THE NEW YORK BOTANICAL GARDEN.

EXPLANATION OF PLATES

PLATE 20

Upper figure (1). *Dermea Cerasi.* In the center photograph of branch showing apothecia (about natural size) with enlarged sketch of an apothecium and stromata above. To the right drawing of ascus and paraphyses. Below sketch of stromata and pycnospores.

Lower figure (2). *Dermea Brenckeleana.* In the center photograph of branch showing apothecia from type material (about natural size). Above large drawing of apothecia. To the left an ascus with spores and paraphyses. Below sketch of stroma and pycnospores.

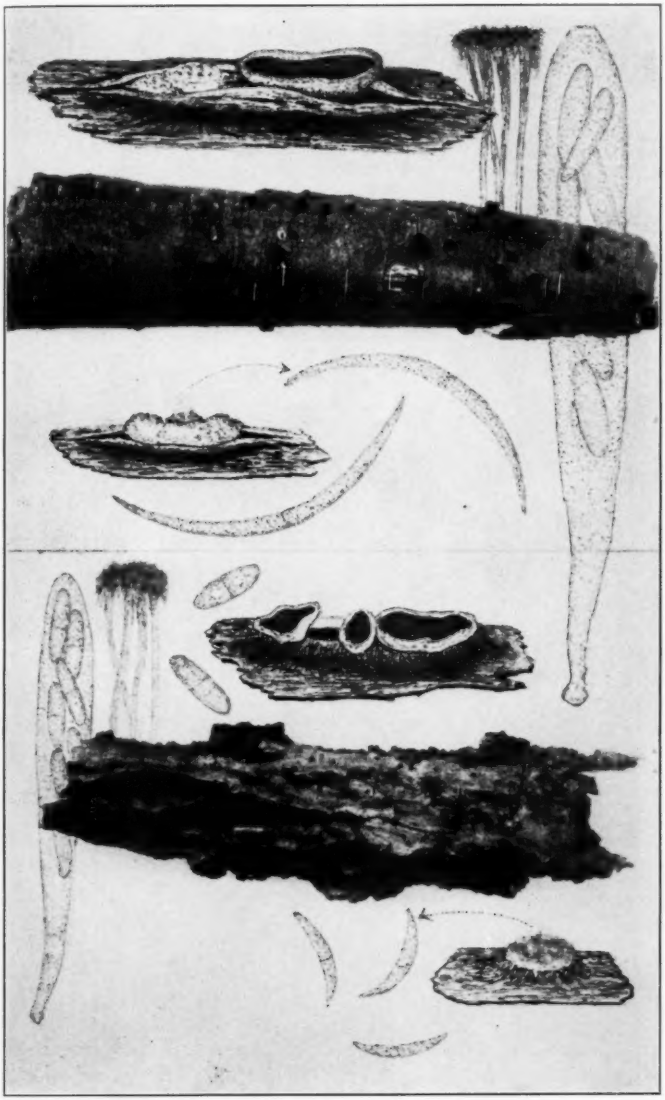
PLATE 21

Upper figure (1). *Dermea Betulae.* Photograph of twigs of *Betula lutea* showing apothecia (about natural size). Above enlarged sketch of apothecia. To the left an ascus with spores and paraphyses. In the center sketch of a stroma and pycnospores.

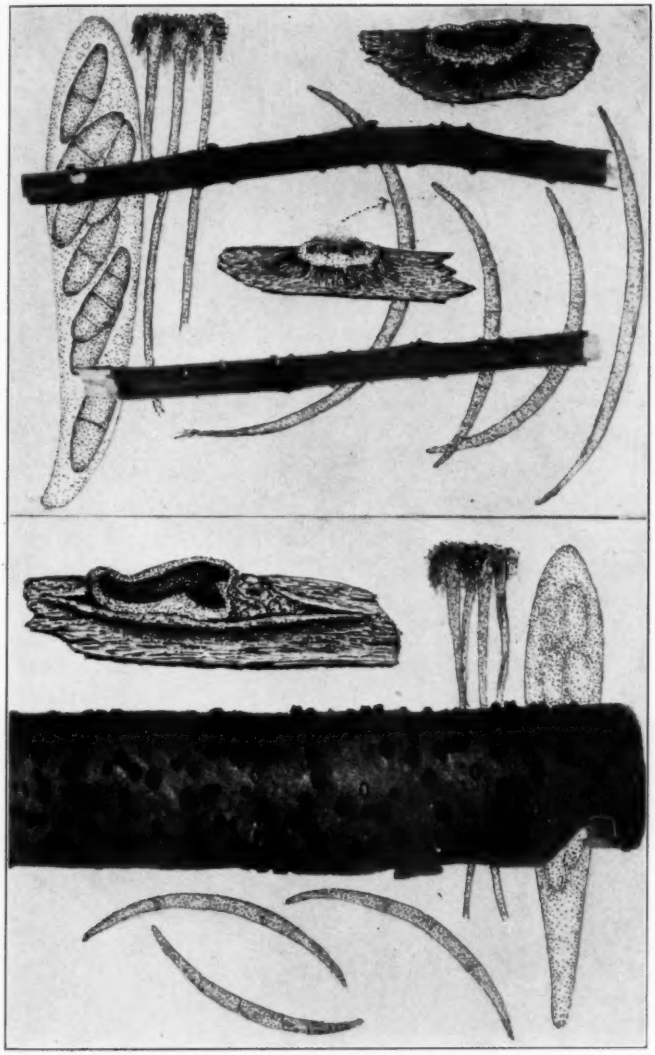
Lower figure (2). *Dermea Betulae.* In the center photograph of branch of *Alnus* showing apothecia (about natural size). Above enlarged sketch of an apothecium and stromata. To the right an ascus with spores and paraphyses. Below pycnospores.

PLATE 22

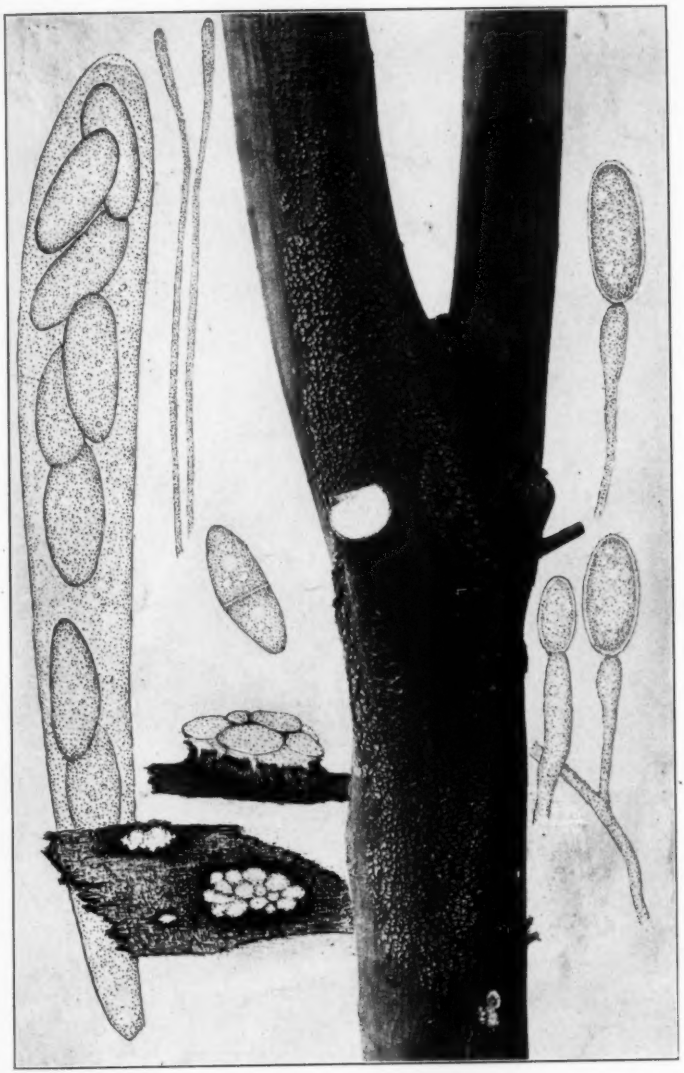
Pezicula carpinea. Photograph of branch showing apothecia (considerably reduced). To the left sketch of apothecia with an ascus with spores and paraphyses. To the left pycnospores in various stages of development.



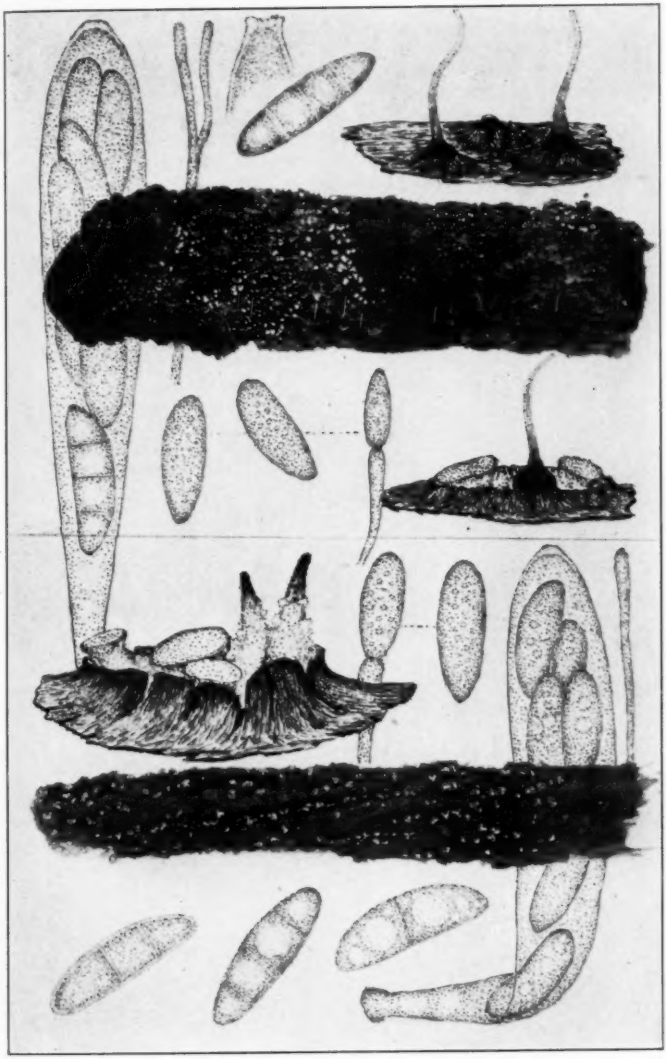
DERMEA CERASI
DERMEA BRENCKELEANA



DERMEA BETULAE



PEZICULA CARPINEA



PEZICULA ACERICOLA
PEZICULA SPICULATA

PLATE 23

Upper figure (1). *Pezicula acericola*. Near the center photograph of bark showing apothecia (about natural size). To the left an ascus with spores and paraphysis and the end of a ruptured ascus. Upper right hand corner sketch of *Sphaeronema acericola*. Lower right hand corner stroma showing both apothecial and conidial stages produced on the same stroma.

Lower figure (2). *Pezicula spiculata*. Near the center photograph of bark showing apothecia and pycnidia (about natural size). Upper left hand corner sketch of stroma showing apothecia and pycnidia with pycnospores. Right hand side an ascus with spores and paraphysis. Below drawing of mature ascospores.

Note: All drawings of ascospores and pycnospores are made with a one inch eye piece and a one-eight objective and drawn with the aid of a camera lucida.

NOTES AND BRIEF ARTICLES

The Lifting Power of a Mushroom

(WITH ONE TEXT FIGURE)

An astonishing phenomenon exhibited by a fungus was witnessed by many persons on August 7 and 8, 1932, when a field mushroom—*Agaricus arvensis*—pushed up through the asphalt pavement in a drive on Wabash College campus, Crawfordsville, Indiana. This drive was paved in 1930. A gravel road bed

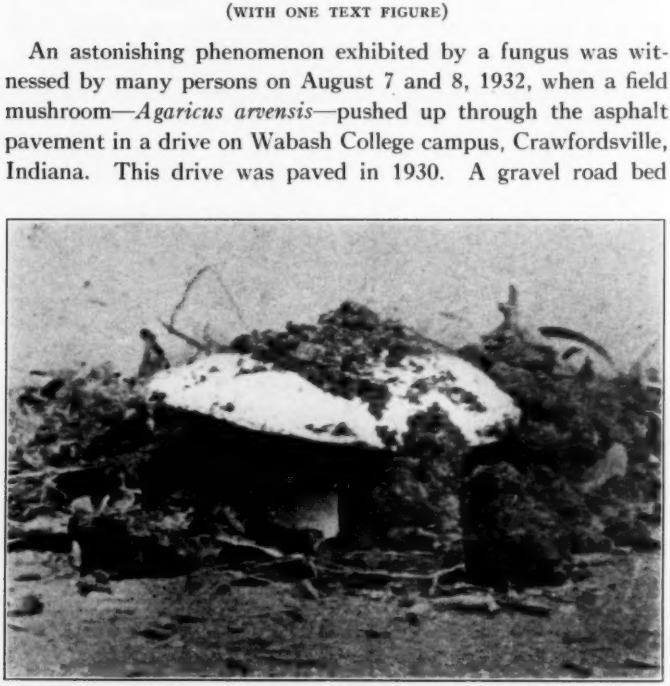


FIG. 1. Lifting power of a mushroom.

topped with about three inches of Kentucky asphalt was subjected to a steam roller. This covering did not seem to phase the mushroom. The photograph shows that it is a normal specimen. The debris on and lying about the mushroom is asphalt and cork from a nearby sycamore tree.—A. R. BECHTEL.

WABASH COLLEGE,
CRAWFORDSVILLE, INDIANA.

Urocystis Heucherae sp. nov.

Sori in the leaves and petioles, more or less distorting them, at first covered with a whitish membrane which when ruptured discloses a black powdery mass of spores; spore balls variable in size, 17–42 μ, averaging 25 μ in diam., mostly spherical or sub-spherical, usually with 3 to 4 spores; the cortex of sterile cells usually completely covering the spores; sterile cells spheroidal, with nearly hyaline walls about 4 μ diam., spores ellipsoidal to rounded-triangular, reddish brown or darker, averaging about 12 μ in diameter.

On *Heuchera parvifolia* Nutt. Collected by A. O. Garrett, Glacier Cirque below Emerald Lake, Mt. Timpanagos, Wasatch Mts., Utah Co., Utah, August 8, 1927 (Garrett Herb. No. *3378*). The *Heuchera* was also badly infected with *Puccinia curtipes* Howe.

Only one other smut, *Urocystis Lithophragmae* Garrett, has been described on a member of the Saxifragaceae which is distinguished from *U. Heucherae* by having smaller, more regular spore balls with from 1–2 spores.

A. O. GARRETT

EAST HIGH SCHOOL,
SALT LAKE CITY, UTAH

TRANSLATION OF TULASNES' CARPOLOGIA

Mycologists owe a debt of gratitude to Doctors A. H. R. Buller and C. L. Shear for making available the English translation by W. B. Grove of the three volumes "Selecta Fungorum Carpologia" of the Tulasne brothers, L. R. and C. Tulasne.

The translation and publication of this work was made possible through the financial assistance of Dr. Howard A. Kelly of Baltimore; James Richardson and Max Steinkopf of Winnipeg; T. B. Macaulay of Montreal; and E. W. Mason of Kew, England. The original copperplate illustrations have been reproduced by collotype.

The Carpologia was one of the outstanding works of its time, its purpose being to demonstrate the pleomorphism of the Ascomycetes. It is invaluable to students of this group. Even though the work was available the Latin was so difficult that it was out of the reach of the average student. Since its translation

and publication entailed a heavy financial outlay, mycologists should coöperate in the enterprise by securing the work for their various institutions. Details may be had from the Editors, Doctors A. H. R. Buller and C. L. Shear.—F. J. SEAVER.

THE MYCOLOGICAL SOCIETY OF AMERICA

CONSTITUTION

(Adopted by the Society, December 28, 1932, at Atlantic City, New Jersey.)

ART. 1. *Name.* The Society shall be known as the Mycological Society of America.

ART. 2. *Membership.*

(1) The Society shall consist of members and may include life members, patrons, honorary members, and corresponding members.

(2) Charter membership in the Society shall consist of the persons who, after the invitation of the Secretary, joined before or during the formal organization of the Society at the Atlantic City meetings in 1932.

ART. 3. *Dues.* The dues for regular members shall be five dollars a year. Any member may become a life member by paying one hundred dollars in one payment or a patron by paying one thousand dollars, and upon election shall have all the privileges of members. Such funds obtained from life members and patrons shall constitute an endowment fund to be used as may be decided by the Council for the support of mycological publications or projects.

Annual dues of five dollars shall include subscription to the official organ of the Society, and shall be payable on or before December 20. Bills for dues shall be sent to the members in October and it will be necessary to discontinue sending the journal to those whose dues have not been paid by December 20.

ART. 4. *Membership and Election of Members.*

(1) All persons interested in the study of the fungi shall be eligible to membership.

(2) Members may be elected at any regular meeting of the Society or in the interim between meetings may be elected by the

Council. Applications for membership must be endorsed by at least one member of the Society.

ART. 5. *Officers.* The officers of the Society shall consist of a President, Vice-president, and Secretary-Treasurer, whose duties shall be those usually performed by such officers. The President and Vice-president shall serve for one year and the Secretary-Treasurer for three years (or until their successors are elected). Any vacancies occurring in the interim between elections shall be filled by the Council.

The Council shall consist of the President, Vice-president, Secretary-Treasurer, and two Councillors. The Councillors shall be elected, one each year, to serve for a term of two years.

ART. 6. *Editors and Committees.* The editors of the official journal of the Society shall be elected by the Council. The President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

ART. 7. *Election of Officers.* The Secretary-Treasurer shall send to each member of the Society in October a ballot for the nomination of officers. All nominations are to be returned by November 15. If any nominations are lacking, the Council shall have power to make them. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot to be sent to each member December 1. Votes shall be mailed to the Secretary-Treasurer and counted by the Council. A plurality vote shall elect.

ART. 8. *Meetings.* An annual meeting shall be held at such time and place each year as the Council may select (usually in connection with the A.A.A.S. meetings). An additional meeting for informal discussion and the carrying out of collecting forays shall be held in the summer or autumn at a time to be selected by the Council. Additional meetings, including special or local meetings for the presentation of papers or the carrying out of forays, may be arranged by the Council at its discretion.

ART. 9. *Divisions.* Branch organizations or units within the Society known as Divisions, may be established on a geographical basis provided formal application, setting forth the reasons for the establishment of the Division, is made to the parent Society and approved by it.

ART. 10. *Journal.* The Society shall adopt or establish a journal which shall serve the Society as its official organ primarily for the publication of mycological papers by its members, for the publication of abstracts of the papers delivered at the annual or other meetings, and for the publication of the report of the Auditing Committee or of other reports, announcements, and business of the Society.

ART. 11. *Amendments.* These articles may be amended by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all of the members at least one month previous to the meeting.

BY-LAWS

1. *Programs.* Programs for annual or other meetings shall be arranged by the Council.

2. *Papers.* Members wishing to present papers at the annual meeting shall submit to the Secretary-Treasurer the substance and conclusions of the papers in a clear and concise abstract of not more than 200 words. These shall be due on or before November 15 and the Secretary-Treasurer shall be authorized to refuse any received after that date. These abstracts will be edited by the editorial committee of the official journal of the Society for subsequent publication in that organ. Members are urged not to submit titles or abstracts unless they expect to attend the meetings. Except by invitation no member shall offer more than two papers at any one meeting, papers of joint authorship being attributed to the author reading the paper.

3. *Associates.* Students and others not yet members of the Society may attend meetings and forays in the status of Associates, provided they are recommended to the Council by a member of the Society and pay a fee of one dollar. Such Associates, as they are not members, shall not have the privilege of voting and shall not receive the official journal of the Society, but shall enjoy the other privileges of the meetings and forays including the right to present one paper on the program.

4. *Auditing.* At each annual meeting the active president shall appoint an auditing committee to audit the accounts of

the Society and of its official publication. An audited statement shall be published in the official organ of the Society.

5. *These rules may be amended* by a majority vote of the members voting at any regular meeting of the Society, provided that suggested amendments have been brought to the attention of the Council of the Society in time to be sent to all the members at least one month previous to the meeting.

NOTE

At the business meeting at Atlantic City the Society voted that the question of the desirability of printing abstracts be referred to the editorial board for report back to the Society at the next annual meeting. This was followed by unanimous approval of a motion that the editorial board be advised that it was the sense of the meeting that abstracts be not published.—H. M. FITZPATRICK, Secretary-Treasurer.

CONTRACT WITH THE NEW YORK BOTANICAL GARDEN

(Accepted by the Society, December 28, 1932, at Atlantic City, New Jersey.)

The Mycological Society of America hereby adopts Mycologia as its official organ on the following terms:

1. Mycologia will continue to be published by the New York Botanical Garden, the editorial policies to be determined by an Editorial Board, consisting of a Managing Editor appointed by the New York Botanical Garden, and five Editors elected by the Mycological Society of America. The term of office of the five elected editors will be five years, except that at the start they will be designated to serve one to five years respectively. One editor will be elected annually, thereafter, to fill the place of each retiring editor.

The six members of the Editorial Board will elect an Editor-in-Chief from among their number. He will be eligible for repeated re-election. Final decision of all questions of editorial policy will be made by him, except that the Managing Editor will have full authority in all matters pertaining to the finances of the journal.

2. All personal subscribers now receiving Mycologia may become members of the Mycological Society of America if they so desire. Institutional subscribers to Mycologia are not to be regarded as members of the Society.

3. All members of the Mycological Society of America in good standing will receive Mycologia. In return the Society will transmit to the New York Botanical Garden, through the Managing Editor four dollars per year for each such member.

4. The New York Botanical Garden agrees to spend on the publication and distribution of Mycologia all funds received from subscriptions, as well as all funds transmitted by the Mycological Society of America. The Garden further agrees to use for these purposes all sums received from the sale of those volumes of the journal which shall be published after this contract is put in force. Earlier volumes remain the property of the New York Botanical Garden. It is understood that the journal will be used by the Garden for exchange purposes as formerly. Should the contract be terminated, it is agreed by the Mycological Society of America that all excess stock of Mycologia then on hand will be regarded as the property of the New York Botanical Garden.

5. The New York Botanical Garden reserves the fourth cover page to be used without charge for the advertisement of its publications, including Mycologia. The other three cover pages will be used by the Mycological Society of America as it may see fit. All sums collected from paid advertising will be expended on the journal.

6. This contract may be altered at any time by mutual agreement of the New York Botanical Garden and the Mycological Society of America. It may be terminated at the end of any calendar year on six months' written notice should it prove unsatisfactory to either party concerned.

7. The contract goes into effect at the beginning of the calendar year 1933.